

New approaches to *Prunus* tree crop breeding

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Abstract

New strategies for *Prunus* improvement, including germplasm and molecular marker development and improved propagation techniques, are described. In germplasm improvement, the introduction of genes from related *Prunus* species conferring several traits including self-compatibility, growth habit, drought resistance, and kernel quality are being pursued. Twin seeds (two embryos within the same seedcoat) have produced seedlings useful for genetic studies. Promising propagation methods include *in-vitro* techniques for the evaluation of plant material and *in-vivo* micrograft techniques that allow the early propagation of high-risk genotypes. In addition, the growth of seedlings in controlled environments, including the induction of an artificial rest period in cold chambers, provides a useful strategy for obtaining vigorously growing plants year round. Molecular markers have also become an essential tool in *Prunus* breeding studies. Different types of molecular markers, including isoenzymes, RFLPs, RAPDs, AFLPs and SSRs, have been employed for the genetic characterization of germplasm, the establishment of genetic relationships between cultivars and species, and the construction of genetic maps. Methodologies for the analysis of marker-assisted selection include the use of mapping populations segregating for desired characters and bulk segregant analysis.

Key words: Fruits, germplasm, propagation techniques, molecular markers.

Introduction

The *Prunoideae*, a subfamily of *Rosaceae*, includes several species producing edible drupes with economic importance. In 2001, worldwide annual production of *Prunoideae* exceeded 28.3 million metric tons, including almost 13.5 million tons of nectarines and peaches (*Prunus persica* (L.) Batsch), 9 million tons of plums (*Prunus domestica* L.), 2.7 million tons of apricots (*Prunus armeniaca* L.), 1.8 million tons of sour and sweet cherries (*Prunus cerasus* L. and *Prunus avium* L. respectively) and 1.3 million tons of almonds (*Prunus amygdalus* Batsch = *Prunus dulcis* (Miller) D.A. Webb) (Table 1)¹. *Prunus* species are characterized by developing only one ovary in which two ovules typically form; one of them degenerates soon after anthesis. The fruit is a drupe where the mature stony endocarp together with the seed forms a propagation unit comparable to a botanical seed surrounded by its protective testa. Breeding practices in *Prunus* must address the challenges resulting from the narrow genetic background of commercial cultivars², a long juvenile period, and the differences in trait expression between juvenile and mature trees³. In the last decade, many techniques such as *in vitro* culture, and the use of molecular markers have become available for *Prunus* crop improvement. These methods are now moving from laboratory evaluation to field application. Simultaneously, different aspects of the utilization of DNA markers, including marker-assisted selection and genome mapping, as well as the impact of altered gene expression on *Prunus* spp., have now been extensively reviewed^{3,6}. This article offers an overview of the current strategies being developed to improve the traits and germplasm in *Prunus* and to optimise breeding efficiency. These strategies include germplasm improvement, propagation techniques and use of molecular markers.

Germplasm Improvement

Related *Prunus* species: The available germplasm in *Prunus* is diverse and the origin and dissemination for several species have been extensively reviewed^{7,11}. It is still possible to find considerable genetic variation for these species mainly in the mountainous areas of Central Asia from the Tian Shan region in China to Kurdistan, including Turkestan, Afghanistan, Iran and Iraq (Fig. 1). However, in cultivated germplasm, a limited gene pool restricts production to specific areas and conditions². The introduction of genes from related species through interspecific hybridisation has been used in several breeding programs throughout the world to develop better-adapted cultivars and rootstocks. Rootstock breeding programs using interspecific hybridisation have introduced useful traits including size control, adaptation to the new environments and pest resistance. Interspecific crosses between *Prunus* species (primarily peach × almond, but also *P. webbii* × peach, and others) have been widely utilized in almond rootstock breeding in France¹², USA¹³, Spain¹⁴, and Yugoslavia¹⁵. The introgression of almond germplasm from related species, including *P. webbii* (Spach) Vieh., *P. argentea* Lam., *P. persica*, *P. bucharica* Korshinsky, *P. mira* Koehne and *P. scoparia* Batal. (Fig. 1) has allowed transfer of several useful traits including self-compatibility, fungal and pest resistance, and frost and drought tolerance^{7,16,18}. *P. davidiana* (Carr.) Frans. has recently been reported to be a source of plum pox virus (PPV) resistance for peach¹⁹, while the introgression of *Prunus mandshurica* (Maxim.) Koehne genes to apricot have improved frost resistance in Eastern and Central European programs²⁰. In almond, the absence of extensive crossing barriers in either the initial hybridisation or subsequent backcrosses demonstrates a direct accessibility of this rich germplasm to breeding^{18,21}. Potential barriers to successful interspecific gene introgression include male sterility, poor germplasm

maintenance, and problems associated with character quality²². The encouraging performance of interspecific hybrids and backcrosses to date, support continuing opportunities for transferring useful traits, including self-compatibility, resistance to important pests and diseases, improvement of seed oil quality, tolerance to aberrant environments, and modified tree architecture and bearing habit¹⁸. International collaborations have further allowed a more thorough evaluation of wild and related germplasm prior to extensive gene introgression²³, and has helped minimize breeding obstacles imposed by quarantine restrictions²⁴.

Multiple embryos: Twin seeds (multiple embryos within the same seed coat) occur spontaneously in several *Prunus* species including peach and almond^{17,25} (Fig. 2). The occurrence of these multiple embryos varies greatly among years and is strongly influenced by environmental conditions. This phenomenon has been mainly studied in peach and in the almond cultivars 'Nonpareil' and 'Mission'. Seedlings from the same twin peach seed are frequently viable and show similar growth habits, though occasionally one of the seedlings show weak growth and develops poorly^{25,26}. Some of these low-vigour plants have been shown to be haploids from which true-breeding dihaploids can be generated²⁵ for genetic studies, hybrid rootstock production, and transformation and regeneration studies. Some of the low-vigour, twin almond seedlings were found to be aneuploids²⁷ and thus, have value for developing near isogenic lines (NIL). A collection of these haploid/aneuploid NILs are presently being developed to aid in genetic (locating genes, transfer of particular chromosomes) and molecular (isolation and sequencing of genes, genetic transformation, etc.) studies as demonstrated by Muehlbauer et al.²⁸ and Young et al.²⁹.

New Propagation Techniques

In vitro evaluation of agronomic traits: The possibility of growing plants and even isolated plant cells in a test-tube under controlled *in vitro* conditions, offers unique opportunities for improving selection efficiency. Advantages include minimizing environment influences, the potential to handle large numbers of individuals in a very small space, and accelerated growth and development³⁰. The increasing availability of other biotechnological techniques (biochemical markers, DNA analysis, genetic transformation, etc.) further complement *in vitro* culture opportunities. Wenzel and Foroughi-Wehr³⁰ reported the utilization in herbaceous species of *in vitro* callus culture for the selection of resistance to environmental stress (freezing tolerance in wheat, salt tolerance in rice) and diseases (*Phytophthora* and *Fusarium* spp.), and herbicide tolerance in tobacco. The application of *in vitro* culture techniques for the selection of horticultural characters, however, may be more difficult. For temperate fruit species, tissue culture propagation has progressed rapidly during the last years. The application of tissue culture techniques as alternative propagation methods has been reported as early as the 1960s. Initially, tissue culture has involved micropropagation and somatic embryogenesis. Axillary shoot production (meristem culture) is the system most frequently utilized to regenerate plantlets by micropropagation techniques^{31,33}. Research in somatic embryogenesis has recently increased in anticipation of more widespread attempts at genetic transformation³⁴. Tissue culture has numerous potential uses for temperate fruit and nut tree species, including propagation

of rootstocks, own-rooted scion cultivars, virus-free stock plants, and elite genotypes^{35,37}. These techniques offer unprecedented opportunities for the evaluation of horticultural traits in breeding programs. Applications have been reported in *Prunus* for the evaluation of the compatibility between cultivar and rootstock³⁸, the resistance to abiotic stress³⁹, and the resistance to biotic stress⁴⁰.

In vivo micrograft: Grafting has been widely used over the centuries for asexual propagation of fruit trees. Micrografts, developed in the 1970s, consist of the grafting of millimetre-size vegetative meristems. Initially, this technique was used for virus elimination in fruit trees. Subsequently, it has been used for the early assessment of rootstock-scion incompatibility, commercial multiplication, virus detection and phytoplasma studies^{41,44}. Micrografts proved to be a useful technique when the early propagation of plant material was desired and to invigorate weak material. Optimum propagation efficiency is achieved through maximizing the different parameters involved in micropropagation and the later growth of the buds⁴⁵. *In vivo* micrografting avoids tissue culture transplant shock when transplanting from sterile *in vitro* conditions. This technique has been employed to recover aneuploids of almond (see above) which occur at low frequencies in sexual embryos with seeds⁴⁶.

Artificial cycle of growth: The growth of woody plants from temperate climates requires a periodic endodormancy, which can be artificially achieved through rest periods in cold chambers^{47,48}. Two cycles of vegetative growth per year can be carried out by employing two 4-month periods of growth in the greenhouse and two 2-month periods of rest in cold chambers. The size of the pots, the periodic renewal of the soil and the control of mites and fungus have played a key role for the successful management of almond seedlings under these controlled conditions⁴⁸. The cold treatments can also be used to control fungi and mites. Seedlings, properly maintained under these conditions, can provide vigorously growing plant tissue (leaves, root tips, etc.) throughout the year. This approach has been used in virus resistance studies, as well as to maintain quarantine conditions (e.g. in the study of dangerous viruses such as the PPV causing sharka disease⁴⁹). Artificial cycles of growth have also been used to invigorate weak genetic material such as the aneuploid seedlings of almond⁵⁰ (see above). Similarly, artificial cold treatments have been successfully utilized in studies of frost tolerance in stone fruit flowers⁴⁷.

Use of Molecular Markers

Characterization and genetic relationships of plant material: Traditionally, characterization and identification of *Prunus* species and cultivars has been based on morphological and physiological traits. However, such traits are not always available for analysis, and are affected by changing environmental conditions. Molecular marker technology offers several advantages over the sole use of conventional markers. Molecular markers developed for *Prunus* also offer a powerful tool to study the evolution of the genome, and for understanding of genome structure and determinants of genetic diversity⁵¹.

Isoenzymes: Isoenzymes were among the first genetic markers to be widely utilized. They have been used for cultivar identification in *Prunus* because of their environmental

stability, their codominant expression, and their good reproducibility. Nevertheless, their utilization is limited by the small number of loci that can be analysed with conventional enzyme staining methods as well as the low variation in some loci. Electrophoretic surveys were particularly useful in characterizing almond^{52,55} and plum⁵⁶ cultivars, because both almond and plum are outcrossing species with high level of isoenzyme polymorphisms. In contrast, peach, a predominantly autogamous species, shows few isoenzyme polymorphisms in spite of its extensive morphological variability^{52,57,59}. Apricot shows intermediate levels of variability^{52,60}, with the predominantly out-crossing non-European populations exhibiting higher isoenzyme variability than the predominantly inbreeding European populations⁶⁰. Isoenzyme analysis has also been used to identify different interspecific hybrids^{58,61-63} and detect phylogenetic relationships among species⁶⁴. More recently, isoenzymes in combination with DNA-based markers were employed to create the genetic maps for woody perennials⁶⁵ and for the genetic characterization of multiple embryos in almond²⁷ (see above).

RFLPs: Restriction fragment length polymorphism (RFLP) markers are based on the differential hybridisation of cloned DNA to bulk DNA fragments from restriction-enzyme digestion. Thus, RFLPs are defined by specific enzyme-probe combinations⁶⁶. RFLP markers are codominant. The primary sources of clones for RFLP mapping are cDNA clones and PstI-derived genomic clones. Genomic clones that represent random sequences may be a poor choice for hybridisation probes because of the large percentage of repeated sequences. RFLPs can detect a virtually unlimited number of markers, thus providing an efficient method for discovering linkages among markers and for constructing genetic maps. This is particularly important in *Prunus* because of the relatively low level of variation typically present in this genus. There are several reports of the use of RFLPs in *Prunus* for map-based selection^{67, 70} and for elucidating the extent of genetic variability⁷¹. However, RFLP analysis has important limitations: it is laborious and time-consuming and it often involves the use of radioisotopes. To overcome some of the difficulties, an alternative called sequence tagged sites (STS) has been developed⁷² which is PCR-based but not requiring radioactive probing.

RAPDs: Random amplified polymorphic DNA (RAPD) markers are based on the PCR amplification of random locations in the genome⁷³. RAPDs are characterized by using arbitrary primers and permit the quick construction of genetic maps and the saturation of specific genomic regions with molecular markers. A single oligonucleotide is utilized for the amplification of genomic DNA. 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 primarily to map construction. RAPD techniques have been successfully used in *Prunus* for identifying cultivars⁷⁴, estimating genetic diversity and assessing possible origins for selected genotypes^{75,76}, and construction of maps. Problems with DNA quality and a general sensitivity to changes in the reaction conditions can hamper the routine utilization of RAPD markers. These difficulties can be overcome by converting RAPDs to sequence-characterized amplified regions or SCARs⁷⁷. In

contrast to RAPD and AFLP (see below) methods, SCAR is a PCR-based method that employs specific primers. These primers amplify single bands corresponding to genetically defined loci. SCARs can potentially be converted into codominant markers and are less sensitive to reaction conditions. Different SCAR markers are being evaluated for marker-assisted selection in *Prunus*, including identification of the *Mal* root-knot nematode resistance gene in Myrobalan plum⁷⁸ and the identification of the *Ff* (flesh adhesion) gene in peach⁷⁹.

AFLPs: 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 PCR⁸⁰. DNA is digested with restriction endonucleases and double-stranded specific adapters are ligated to the ends of the DNA fragments to obtain template DNA for subsequent amplification by PCR. The subset of amplified fragments is then analysed by denaturing PAGE to generate the fingerprint. AFLP has a number of advantages over the RAPD technique: more loci analysed per experiment and better reproducibility of banding patterns resulting from the higher specificity of primer annealing to complementary adapters. Powell et al.⁸¹ found that AFLPs had a much higher multiplex ratio (number of polymorphic products per "reaction") than other molecular marker systems. Consequently, AFLPs also shows a higher marker index. These markers have been mainly used in *Prunus* for genetic mapping (see below) and molecular characterization and estimation of genetic diversity among apricot cultivars⁸².

SSRs: PCR-based, simple sequence repeat (SSR) markers (microsatellites) are becoming the marker of choice for fingerprinting and genetic diversity studies for a wide range of plants⁸³. Because of their high polymorphism, abundance, and codominant inheritance, they are well suited for the assessment of genetic variability within crop species, and of the genetic relationships among species⁸¹. Recently, SSR primers generated from different *Prunus* species have been reported in peach⁸⁴⁻⁸⁸, cherry^{89,90}, and almond⁹¹. These markers are being used for the molecular characterization and estimation of genetic diversity among peach and almond cultivars (Figs. 3 and 4)^{50,87,88}, sweet cherry cultivars⁹², and apricot cultivars⁹³. SSRs have been employed in the genetic characterization of multiple embryos in almond²⁷ and genetic mapping (see below). Studies of genetic diversity and genetic relatedness utilizing molecular markers thus offer unprecedented opportunities for improving *Prunus* breeding efficiency when using either established cultivars or interspecific germplasm. Similar opportunities have also been demonstrated for the analysis of chloroplast DNA⁹⁴. SSRs are currently being employed for the molecular characterization and estimation of genetic diversity and the genetic relationships among peach and almond cultivars and related *Prunus* species^{95,96} (Fig. 5). In addition, recent studies have had shown promise for analysing variation of internal transcribed spacers (ITS) in nuclear ribosomal DNA⁹⁷ and chloroplast DNA⁹⁸.

Genetic Mapping: Several intraspecific and interspecific *Prunus* maps have been developed using different types of molecular markers. The utilization of PCR-based markers has made mapping and tagging of a wide range of traits possible^{3,99}. The analysis of cosegregation among markers greatly facilitates

linkage analysis between markers and major or quantitative loci controlling horticulturally important traits. Different research groups have released linkage maps using isoenzymes, RFLPs and RAPDs in peach^{67,69,100–106}, almond^{68,91,107}, sweet cherry¹⁰⁸, sour cherry⁷⁰, apricot¹⁰⁹, and peach × almond hybrids^{110,111}. Similarly, AFLPs allow detection of a higher level of polymorphism in peach than isoenzymes, RFLPs or RAPDs^{69,88}. SSR has also been used for mapping in peach^{88,112} and almond^{91,113}. The first genetic linkage map for a *Prunus* rootstock population was constructed using AFLPs¹⁰⁴. The similar order of markers observed in different *Prunus* maps suggests a high level of synteny within the genus^{113,114}. This homology among *Prunus* species partly explains the low level of breeding barriers to interspecific gene introgression (see above) and supports the opportunity for successful gene transfer between closely related species.

Marker-assisted selection: Marker-assisted selection (MAS) is emerging as a very promising strategy for increasing selection gains¹¹⁵. 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 programs¹¹⁶. Marker loci linked to major genes can be used for selection, and sometimes is more efficient than direct selection for the target gene¹¹⁶. Selection by molecular markers is particularly useful in fruit and other tree crops 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^{5,6}. The principal approach for the analysis of marker-trait association in *Prunus* is the use of mapping populations segregating for the characters of interest. The different linkage maps developed in *Prunus* (peach, almond, cherry, and apricot) include markers associated with several traits of horticultural value³. Mapping quantitative characters by identifying quantitative trait loci (QTL) is also becoming an important tool in tree breeding. QTLs are generally recognized by comparing the degree of covariation for polymorphic molecular marker and phenotypic trait measurements. Important characters and QTLs that are presently being mapped in stone fruits include the control of flower (bloom time, self-incompatibility, pollen-sterility, double-flowers), fruit (shape, pubescence, flesh colour, acidity and sweetness), leaf (red vs. green colour) and tree traits (pillar or weeping architectures), and resistance to various pests and diseases (root-knot nematodes, powdery mildew, leaf curl, Plum pox potyvirus, etc.)^{69,103,106,107,109,112,117–120}. The high degree of genome synteny observed among *Prunus* species¹¹⁴ should also facilitate the successful transfer of sets of markers and coding sequence among species. Bulk segregant analysis (BSA), where two pooled DNA samples are formed from plant sources which have similar genetic backgrounds but differ in one particular trait, is another promising approach for the analysis of molecular marker-horticultural trait association. This method also makes possible the identification of markers linked to the trait of interest¹²¹. A strategy combining different markers with bulk segregant analysis was used to identify markers linked to loci of specific characters in peach and peach × almond crosses¹⁰², RAPD markers flanking the red-leaf (Gr) and malate dehydrogenase loci in the NC174RL × Pillar and Marsun × White Glory F2 peach families¹⁰⁰, and three RAPD markers associated with a delayed bloom gene in almond¹²⁰. Also, it has facilitated the study of self-incompatibility and

male sterility in almond¹²². BSA and RAPD analysis were recently utilized to distinguish markers linked to the *Mal* gene, a major dominant gene that controls a wide-spectrum resistance to root-knot nematodes in *Myrobalan plum*⁷⁸, as well as markers linked to resistance to PPV in apricot¹²³ and to ring nematode in peach¹²⁴.

Apart from isoenzymes, RFLP, RAPD, AFLP, and SSR, other markers being used in the development of marker associated traits, are those based on single point mutations (SNPs) and those obtained from either cDNA sequences (ESTs) or databases (Cloned Gene Analogs, CGAs)^{125,126}. The first genetic linkage map for a *Prunus* rootstock population was constructed using AFLP technology¹⁰⁴ and, simultaneously, two genes that control resistance to root-knot nematodes, *Mi* and *Mij*, were mapped and tagged. The conversion of the AFLP marker linked to the *Mij* (a gene required for resistance to *Meloidogyne incognita* and *javanica*) locus to STS proved to have practical application for germplasm screening and for breeding peach rootstocks for resistance to root-knot nematodes¹²⁷. An extensive application of molecular marker assisted selection is taking place in the manipulation of self-compatibility in *Prunus*. Most species are predominantly self-incompatibles. Self-incompatibility is of the gametophytic type and acts to prevent self-fertilization. This character is controlled by a single locus with multiple codominant alleles^{128,129}, and is expressed within the styles of flowers as *S*-RNases glycoproteins^{130,132} which are responsible of the subsequent inactivation of self-pollen tube growth. Almond self-incompatibility alleles (*S*-alleles) were initially identified in the field through controlled crosses with a series of known *S*-genotypes¹⁷. More recently, molecular methods have been developed in two areas: identification of stylar *S*-RNases by electrophoresis in vertical polyacrylamide gels^{131,133,134}, and the amplification of specific *S*-alleles using appropriately designed primers for PCR and electrophoresis in horizontal agarose gels^{135–137}. This technique is being routinely used for the identification of cross-incompatibility groupings for current almond cultivars and for efficiently breeding self-compatibility into new cultivars¹³⁸ (Fig. 6).

Conclusions

In conclusion, the typical long generation time along with the difficulties in generating large segregating progeny populations have frustrated the development and testing of new, often molecular-based, breeding strategies. This same limitation, however, makes new strategies that improve breeding efficiency especially valuable to tree crops. Most *Prunus* tree crops, because they are vegetatively propagated, have a unique advantage over other agronomic crops since desirable, unique gene combinations can be ‘captured’ by clonal propagation. Additional advantages promoting the utilization of these new technologies to *Prunus* tree crop improvement include a small genome size, high levels of synteny between genomes, and a well-established international network of cooperation among researchers.

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Table 1. *Prunus* production in thousand of tones (MT) in the world during the years 1998-2001.

	1998		1999		2000		2001	
	MT	%	MT	%	MT	%	MT	%
Peaches and Nectarines								
WORLD	11,494	100.0	13,268	100.0	13,439	100.0	13,495	100.0
China	3,237	28.1	3,983	30.2	3,974	29.5	4,126	30.5
Italy	1,425	12.4	1,766	13.3	1,655	12.3	1,680	12.4
USA	1,292	11.2	1,394	10.5	1,421	10.5	1,355	10.0
Spain	910	7.9	986	7.4	1,127	8.3	1,030	7.6
Plums								
WORLD	7,679	100.0	8,502	100.0	9,102	100.0	9,051	100.0
China	3,161	41.1	3,919	46.1	3,941	43.3	4,145	45.7
USA	507	6.6	668	7.8	825	9.1	585	6.4
Romania	404	5.2	361	4.2	471	5.1	430	4.7
Germany	338	4.4	388	4.5	570	6.2	387	4.2
Almonds								
WORLD	1,320	100.0	1,392	100.0	1,250	100.0	1,335	100.0
USA	393	29.7	377	27.1	318	25.5	385	28.8
Spain	220	16.6	279	20.0	223	17.8	257	19.2
Italy	87	6.6	103	7.4	104	8.3	105	7.8
Iran	111	8.4	95	6.2	89	7.1	87	6.5
Apricots								
WORLD	2,508	100.0	2,684	100.0	2,712	100.0	2,681	100.0
Turkey	540	21.5	500	18.6	500	18.4	500	18.6
Iran	243	9.6	240	8.9	230	8.4	225	8.3
Italy	135	5.4	212	7.9	204	7.5	199	7.3
Spain	163	6.5	147	5.5	128	4.7	159	5.9
Cherries								
WORLD	1,624	100.0	1,766	100.0	1,876	100.0	1,803	100.0
Iran	229	14.1	228	12.9	220	11.7	218	12.1
USA	178	10.9	196	11.1	187	10.0	208	11.5
Turkey	195	12.0	200	11.3	200	10.6	200	11.1
Germany	122	7.5	156	8.8	169	9.0	133	7.3



1. *P. argentia*
2. *P. bucharica*
3. *P. fenziana*
4. *P. davidiana*
5. *P. persica*
6. *P. scoparia*
7. *P. webbii*
8. *P. dulcis*
9. *P. armeniaca*
10. *P. manshurica*
11. *P. japonica*
12. *P. avium*

Figure 1. Map of Asia showing origins for different *Prunus* species.

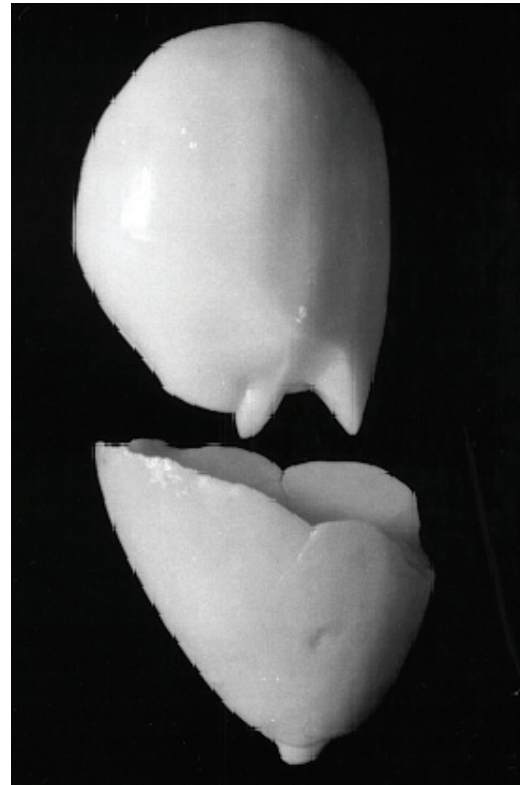


Figure 2. Detail of twin embryos in an almond seed.

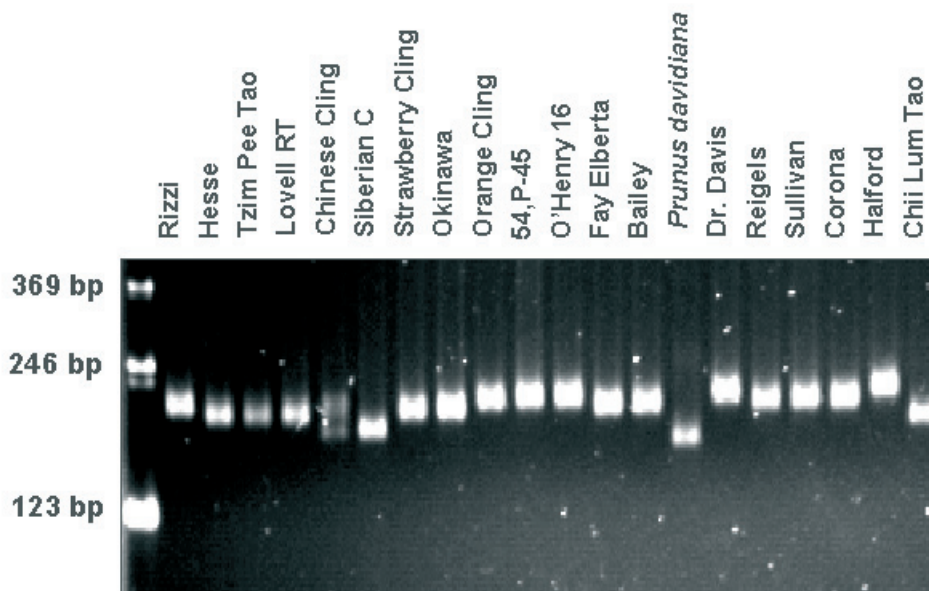


Figure 3. Application of molecular markers in the study of genetic relationships in peach cultivars. Allelic segregation of a SSR marker among peach cultivars and the related *Prunus* species *P. davidiana*.

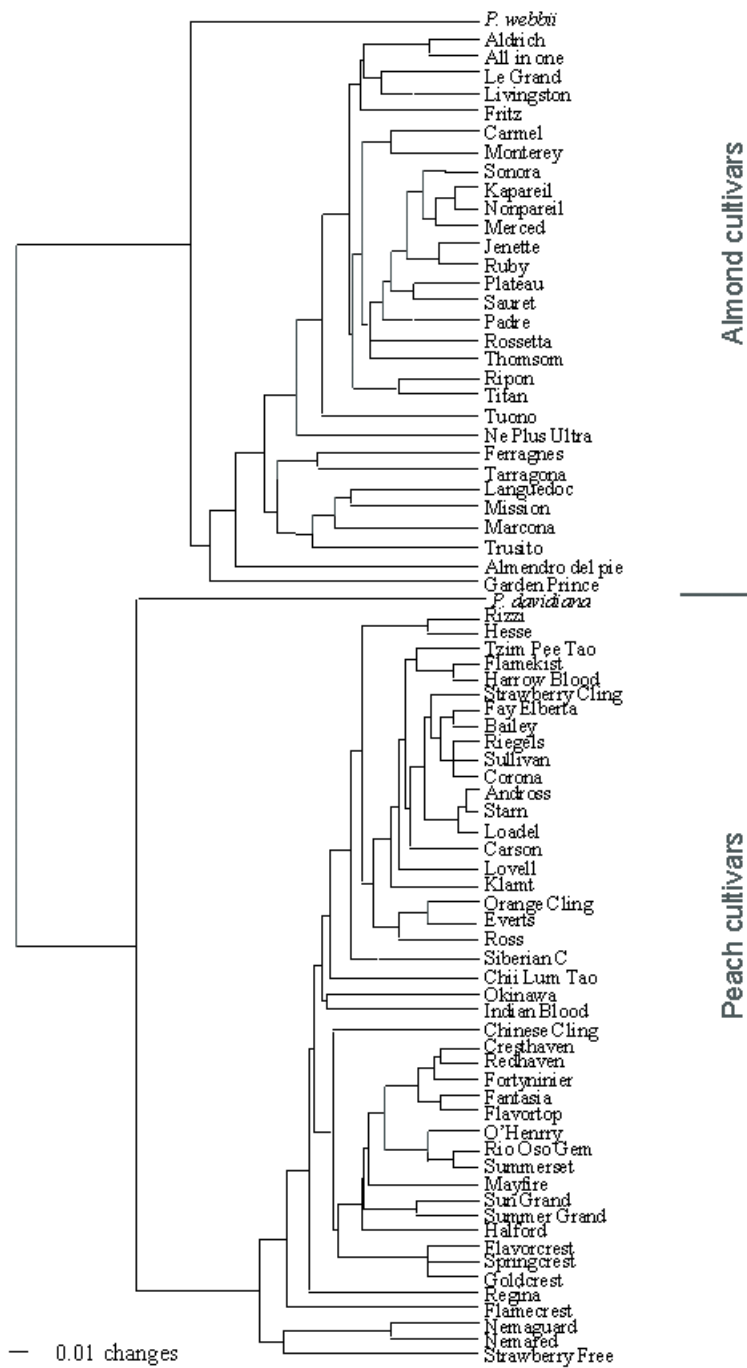


Figure 4. Genetic relationship among peach and almond cultivars obtained by a study with SSR markers. Dendrogram obtained by Neighbour Joining cluster analysis⁵⁰.

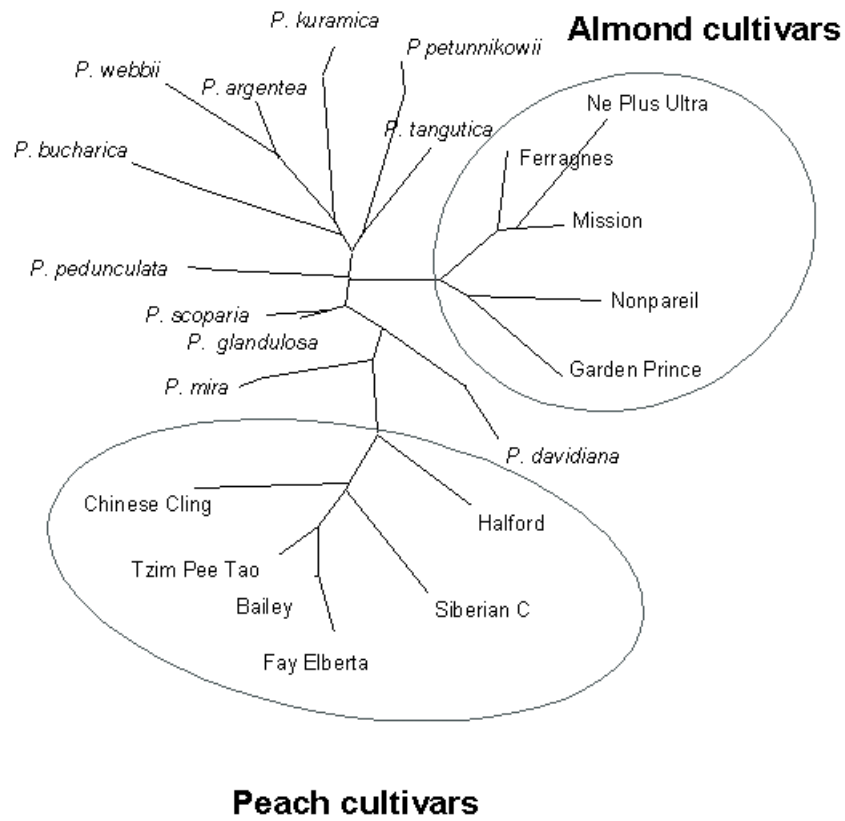


Figure 5. Genetic relationship among peach and almond cultivars and related *Prunus* species obtained by a study with SSR markers. Unrooted dendrogram obtained by Neighbour Joining cluster analysis⁹⁵.

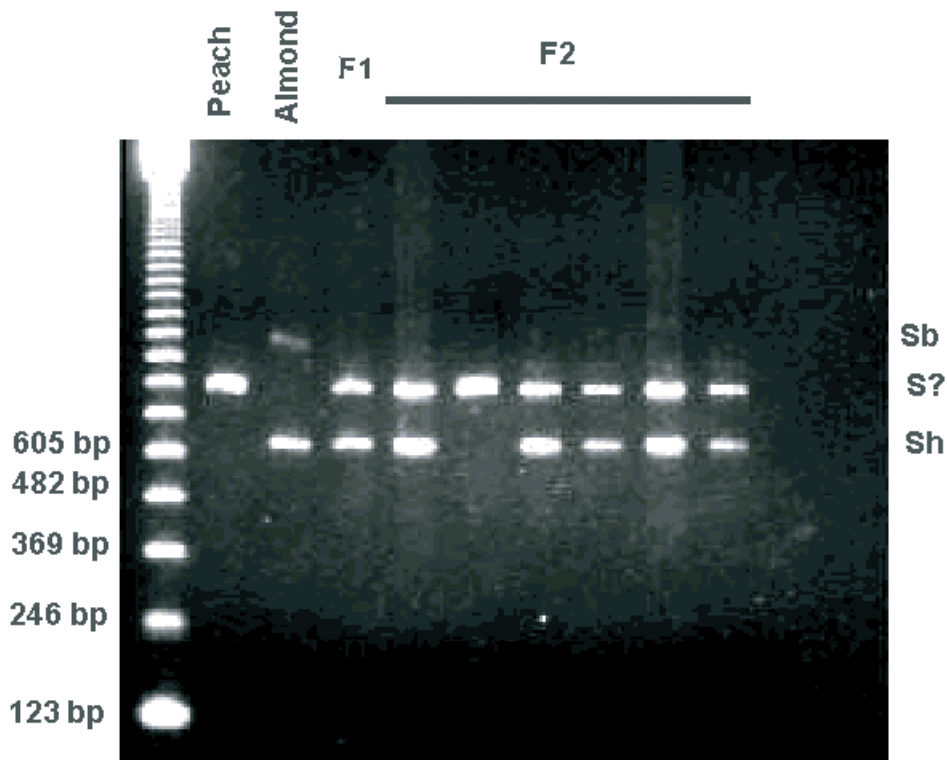


Figure 6. Application of molecular markers in assisted selection in *Prunus* breeding programs. Self-incompatibility alleles determination by PCR in an interspecific cross almond × peach.