12 Pistachio

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12.1 Introduction

12.1.1 History of the Crop

Pistachio (*Pistacia vera* L.), a deciduous, dioecious, and wind-pollinated tree species, is a member of the family Anacardiaceae. This family includes other known species such as cashew (*Anacardium occidentale* L.), mango (*Mangifera indica* L.), ambarella (*Spondias dulcis* Forst.), purple mombin (*Spondias purpurea* L.), poison ivy and poison oak (*Toxicodendron* spp.), pepper tree (*Schinus* spp.), or sumac (*Rhus* spp.). Although pistachio is widely cultivated in Mediterranean countries, its probable origin is central and southwestern Asia. The most complete surveys of the current range of wild pistachio trees were made by Whitehouse (1957), who traveled to southwestern Asia in the late 1920s, and by Zohary (1952, 1973). These two authors note that *P. vera* grows wild in the low mountains and foothills of the semi-desert region of south-central Asia. The range extends from northeast Iran and northern Afghanistan to western Tien-Shan and the Karatau mountains through Turkmenistan, Uzbekistan, Tajikistan, Kazakhstan and Kyrgyzstan (Kayimov et al. 2001). East of the Karatau range, in the central Tien-Shan range, wild pistachio trees exist in only a few, separate, small areas, and the species also grows in Baluchistan (western Pakistan) (Anwar and Rabbani 2001). Historical records, however, tell of pistachio trees growing in places where none exists today, and the present distribution has been influenced by exploitation of pistachio trees by local human populations, who used them as a source of fuel and heavy pasturing of cattle, preventing natural renewal (Whitehouse 1957).

The presence of pistachio nuts in archeological excavations provides evidence that pistachio has long been associated with human activities, although these reports do not always indicate if the nuts found are from *P. vera* or from closely related *Pistacia* species such as *P. palaestina* or *P. atlantica* (Hormaza et al. 1994a). Pistachio cultivation is very ancient and probably started in areas close to wild pistachio stands, likely from seedlings obtained from the best wild trees (Whitehouse 1957). Remnants of true pistachio nuts dated from the sixth millennium BC have been found, east of the Zagros mountains, in Shortughai, Afghanistan (Wilcox 1991) and in Yahya in the Soghum valley of southeastern Iran (Prickett 1986), two places that were situated close to wild pistachio stands. From its presumed center of origin, pistachio cultivation was extended within the ancient Persian Empire from where it gradually expanded westward. In fact, according to Joret (1976), the name pistachio seems to derive from the word pista-pistak in the ancient Persian language Avestan. In Assyria, about the tenth century BC, the Queen of Sheba monopolized the limited crop of nuts for her exclusive use and that of her guests (Whitehouse 1957). Pistachio trees were also planted in the gardens of the king Merodach-Baladan of Babylon around the eighth century BC (Brothwell and Brothwell 1969). Pistachio nuts, called botnim in Hebrew, are mentioned in the Bible (Genesis 43:11) as precious gifts carried from Canaan to Egypt by the sons of Jacob (Zohary 1982). In the second century BC, Nicander found pistachios in Susa, a village in southwestern Iran close to the current border with Iraq (Joret 1976). In the first century BC, Poseidonius finds cultivated pistachios in Syria which misled Greek and Roman writers to consider Syria as the site of origin for pistachio (Joret 1976), a misconception that persisted until recent times (Zohary 1973). Pliny wrote in his *Natural History* that pistachio was introduced into Italy from Syria by the Roman consul in Syria, Lucio Vitello, at the end of the reign of the emperor Tiberius early in the first century AD (Bonifacio 1942). From Italy it was introduced into Spain by Flavius Pompeius, and, probably at that

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time, to other Mediterranean regions of Southern Europe, North Africa, and the Middle East (Lemaistre 1959). Pistachio cultivation was also extended eastward from its center of origin and it was reported in China around the tenth century AD (Lemaistre 1959). More recently, its culture has begun in Australia and in California.

12.1.2
Botanical Description

Pistachio (*Pistacia vera* L.) is a diploid ($2n = 30$) (Zohary 1952; Ila et al. 2003) member of the Anacardiaceae. This virtually cosmopolitan family in the Sapindales/Rutales (Wannan and Quinn 1991) comprises about 70 genera and over 600 species (Mitchell and Mori 1987). The most widely accepted classification divides the family into five tribes: Anacardieae, Rhoeae, Semecarpeae, Spondiadeae, and Dobineae (Mitchell and Mori 1987; Wannan and Quinn 1991), with *Pistacia* belonging to the tribe Rhoeae. Zohary (1952) considers the genus to comprise 11 species divided into four sections (*Lentiscella*, *Eu Lentiscus*, *Butmela* and *Eu Terebinthus*, where *P. vera* is included), although some authors recognize as many as 15 species (Whitehouse 1957). Except the North American species *P. texana* and *P. mexicana*, *Pistacia* species are distributed mainly within the Mediterranean region, Western and Central Asia and the Middle East.

The pistachio is a small to medium sized, bushy, deciduous tree which grows slowly to a height of about 6–9 meters with a single or several trunks. Leaves are compound-pinnate, hairy when young and glabrous when old, with three to five oval leaflets. Pistachio is dioecious and both the staminate and pistillate inflorescences are panicles formed in the axils of the previous year's growth, consisting of up to several hundred individual flowers (Crane and Iwakiri 1981). Both types of flowers are apetalous and wind is the pollinating agent. *P. vera* shows perfect dioecy since mature pistillate flowers have no trace of stamens and mature staminate flowers lack any evidence of female structures (Wannan and Quinn 1991). However, stamen and carpel primordia are initiated in both male and female flowers, but the development of organs of the opposite sex becomes arrested at the primordial stage (Hormaza and Polito 1996). Mature pistillate flowers consist of two to five tepals and a pistil with three stigmas. The commercial pistachios are known as nuts, but the pistachio fruit is indeed a drupe with a fleshy exocarp and mesocarp (hull) and a hard, bony, dehiscent endocarp (shell) that splits longitudinally along its suture beginning at the apex when the fruit has ripened. The pistachio nuts of commerce comprise the endocarp (shell) and the edible kernel, which has a papery seed coat, which color ranges from yellowish to green. Although the fleshy hull loosens at maturity, it has to be removed from the nut in processing either by hand or mechanically.

12.1.3
Economic Importance

Although some species other than *P. vera* produce seeds that are eaten or used for oil and soap production by local populations in their native ranges (Hepper 1992), *P. vera* is the only commercially important species in the genus *Pistacia* (Whitehouse 1957). Among the nut tree crops, pistachio tree ranks sixth in world production behind almond, walnut, cashew, hazelnut and chestnut (Mehlenbacher 2003). Total pistachio world production has reached about 650,000 tons (2002–2004 average) and the main world producer is Iran with more than 300,000 tons followed by Turkey, USA and Syria (Faostat 2004). Pistachio nuts are an excellent source of vegetable protein, with a high arginine and unsaturated fat content, mainly monounsaturated, as well as phytosterols. Pistachios also contain appreciable levels of copper, magnesium, phosphorus and calcium as well as many vitamins such as vitamin E and folic acid (Favier et al. 1995).

Most pistachio cultivation is based on clonally propagated scion cultivars grafted onto seedling rootstocks of the same species, or of other *Pistacia* species or hybrids. Different rootstocks are used in the different growing areas, thus, *P. atlantica*, *P. integerrima* and hybrids *P. atlantica* × *P. integerrima* are the main rootstocks in California, *P. vera* seedlings are used in Turkey, while *P. mutica*, *P. khinjuk* as well as seedlings of *P. vera* are the main rootstocks used in Iran. In areas where wild forms exist, wild *P. vera* individuals are occasionally grafted in situ with selected genotypes (Zohary and Hopf 1988). The few cultivars of *P. vera* described, probably less than 100 worldwide, are thought to be derived from only a few primitive varieties (Maggs 1973) resulting in a high degree of genetic vulnerability. These cultivars are relatively few generations removed from their wild ancestors and usually only a few cultivars are grown in a given pistachio producing area. The main cultivars grown in Iran are Ohady, Kaleh ghochi, Ahmad

Aghai, Badami Zarand, Rezaii and Pust piazi (Esmailpour 2001); in Turkey, Uzun, Kirmizi, Halebi, Siirt, Beyazben, Sultani, Değirmi and Keten Gömleği (Ak and Açar 2001); and in Syria, Ashoury, Red Oleimy and White Batoury (Hadj-Hassan 2001). The California pistachio industry relies almost exclusively on two seedling selections: 'Kerman', a nut-producing female cultivar, and 'Peters', a male cultivar used as a pollinizer.

The pistachio is a xerophitic tree that requires long and hot summers and moderately low winter temperatures. These conditions are met in Iran, Turkey and California but some pistachio producing countries from the Mediterranean basin like Syria and Tunisia usually require varieties with lower chilling requirements. Similarly, in regions with spring frosts or high summer humidity and rainfall, the pistachio trees may suffer during blooming and can be affected by diseases not found in dry areas (Crane 1984). Pistachios are adapted to a variety of soils, but commercial production is best on well-drained, deep, light, sandy loams with high lime content. Although pistachio can thrive in arid conditions, yields in non-irrigated conditions are much lower than in irrigated conditions such as California. Several diseases and pests can affect pistachios (Holtz 2002) although their incidence is variable depending on the cultivar and environmental conditions.

12.1.4 Breeding Objectives

The main tasks carried out in the different existing pistachio collections include characterization and evaluation trials (Caruso et al. 1998). Only recently, a few breeding programs have started in different places, like California (Parfitt et al. 1995; Chao et al. 1998, 2003), Spain (Vargas et al. 1987, 1993, 2002; Batlle et al. 2001) and Turkey (Atli and Kaska 2002). As in other tree-crop species, traditional breeding progress has been slow and many of the selections are still in experimental collections.

Selection and breeding in male pistachio trees is directed towards obtaining pollinators that produce a large amount of viable pollen, with an overlap in blooming with the female cultivar of interest (Martinez-Palle and Herrero 1994). Most of the breeding effort has been focused on the fruiting related problems of female pistachios. The main traits of interest in current pistachio breeding programs include:

- Increasing the percentage of split shells. Split shells make pistachio nuts more attractive to consumers since the kernels can be extracted with the fingers avoiding cracking. The percentage of split shells depends on the cultivar and on environmental conditions (Crane 1984).
- Reduction in the number of blank or unfilled nuts. Blanks are fruits without kernels resulting mainly from embryo abortion and subsequent fruit development (Crane 1973). The extent to which blanks occur varies upon cultivar and rootstock and year to year (Crane 1984). It has been observed that, in blank nuts, the shell does not split, indicating the involvement of the seed in shell dehiscence.
- Increased and regular yields trying to diminish alternate bearing. Alternate bearing is a common problem in most pistachio cultivars, resulting in a heavy crop one year followed by little or none the following year. This problem is observed in other fruit crops and, in pistachio, it seems to be the result of the premature abscission of inflorescence buds during a heavy crop year (Crane and Nelson 1971) although the physiological causes are still not clear (Roussos et al. 2003).
- Vegetative propagation. Pistachio rootstocks are produced from seed. The genetic variation found in seedling rootstocks results in a great variability in the performance of the grafted cultivar affecting diverse traits such as fungal resistance, shell splitting, blank nut production or yield. Consequently, a greater uniformity is desirable and it could be obtained by using vegetative propagated rootstocks (Crane 1984). Important advances have taken place in micropropagation (Parfitt and Almehdi 1994) and micrografting (Onay et al. 2004a). Recently, clonal propagation from leaf cuttings of the rootstock UCB-1 (hybrid between *P. atlantica* and *P. integerrima*) widely used in California has been also reported (Almehdi et al. 2002).
- Increase in nut size, quality and appearance. As in other crops, the improvement of quality traits are becoming important breeding objectives towards a greater acceptance of the product by the consumer and to reduce the number of undesirable fruits.
- Later flowering. Late flowering in regions with late frosts is a desirable breeding and selection trait to avoid flower damage that would affect production and to avoid spring fungal infections.
- Resistance to both aerial and soil fungal diseases. Several fungal diseases affect pistachio but their impact is highly variable depending on the cultivar

and the environmental conditions. The most important include: panicle and shoot blight (caused by *Botryosphaeria dothidea*), botrytis blossom and shoot blight (caused by *Botrytis cinerea*), alternaria late blight (caused by *Alternaria alternata*), Verticillium wilt (caused by *Verticillium dahliae*), Armillaria root rot (caused by *Armillaria mellea*), Schizophyllum wood decay (caused by *Schizophyllum commune*), Sclerotinia shoot blight (caused by *Sclerotinia sclerotiorum*), Phomopsis shoot blight (caused by *Phomopsis spp.*) and Septoria leaf and fruit spot (caused by *Septoria spp.*) (Michailides et al. 1995; Holtz 2002).
– Other interesting traits in rootstock breeding include resistance to nematodes and salinity.

Although a few exceptions have been described in various *Pistacia* species (Ozbek and Ayfer 1958; Crane 1974; Kafkas et al. 2000) dioecy is the norm in pistachio. Dioecy represents an inconvenience for pistachio breeding because pistachio seedlings need between five to eight years to reach reproductive maturity and both sexes are phenotipically indistinguishable at the seedling stage (Hormaza et al. 1994b). However, molecular methods (see below) can facilitate breeding and selection by enabling screening for gender at the seedling stage, thereby simplifying the breeding of male and female plants for different objectives, with savings of time and economic resources.

12.1.5 Classical Breeding Achievements

Breeding and introducing new pistachio varieties is of great interest for the pistachio industry in different areas of the world. The use of a single variety, like 'Kerman' in the US, makes pistachio production very vulnerable to new diseases, and limits the possibility of extending the ripening season. Additionally, due to the little breeding efforts made until recently, most of the varieties used today have low levels of the desired characteristics like percentage of split nuts, number of blanks or exhibit an extreme alternate bearing. This situation limits production and, therefore, breeding towards the improvement of these characteristics can greatly improve yields. Due to the relatively recent initiation of pistachio breeding programs and to the long time required to achieve results in fruit tree species, the progress obtained in this direction following classical selection breeding approaches is slow. The most advanced pistachio breeding programs are now evaluating advanced selections from breeding crosses. This is the case of the program initiated at the University of California-Davis in the USA (Parfitt et al. 1995; Chao et al. 1998, 2003), at the IRTA Mas Bové in Spain (Vargas et al. 1987, 1993, 2002; Batlle et al. 2001) and at the Pistachio Research Institute in Gaziantep in Turkey (Mehlenbacher 2003). In other countries like Iran, Turkey, Israel or Australia, current pistachio genetic improvement involves evaluating cultivars, local seedling populations and species (Mehlenbacher 2003). In Turkey, monoecious *P. atlantica* genotypes are being investigated to determine the mechanism and inheritance of sex determination in the species (Kafkas 2002). Regarding disease resistance, the impact of *Verticillium* wilt on the susceptible species *P. atlantica* and *P. terebinthus* is now minimized by using *P. integerrima* hybrids, resistant to this fungus, as rootstock in infected soils (Morgan et al. 1992). Also, heritable resistance to *Alternaria* (Chao et al. 2001) and to *Botryosphaeria* (Parfitt et al 2003) has been identified in pistachio progenies.

12.2 Marker-Assisted Breeding

No genetic maps have been released so far in pistachio, although some advances have been made in the use of molecular tools for germplasm screening and breeding for specific traits. Results on genetic transformation of pistachio are also not yet available, although efficient somatic embryogenesis protocols have been reported (Onay et al. 1995, 1996, 2000, 2004b).

12.2.1 Germplasm Screening

Several studies have been conducted in pistachio concerning intra- and inter-specific genetic relationships, patterns of inheritance, or breeding histories. As in other fruit tree species, identification of pistachio cultivars has been traditionally carried out through pomological, morphological and horticultural traits (Zohary 1952; Grundwag and Weker 1976; Lin et al. 1984), and the consensus on those traits has allowed the release of descriptors for pistachio (IPGRI 1997).

More recently, as in other fruit tree species (Wünsch and Hormaza 2002), different molecular markers have been used to fingerprint pistachio cultivars and to perform genetic diversity studies. Molecular characterization of *Pistacia* cultivars and species was initially carried out using isozymes (Loukas and Pontikis 1979; Dollo 1993; Barone et al. 1993, 1996; Rovira et al. 1995; Vargas et al. 1995). However, insufficient isozyme polymorphism among closely related cultivars limits their usefulness for fingerprinting and genetic diversity studies. In the last two decades efforts have been dedicated to obtain a more objective identification of genotypes with the use of DNA-based molecular markers. Initial work on molecular identification of pistachio using DNA markers was carried out by Hormaza et al. (1994a) and Dollo et al. (1995). They examined 15 pistachio cultivars with 33 RAPD primers and selected 14 primers that produced 143 amplification fragments, 37 of them being polymorphic. UPGMA cluster analysis grouped the cultivars according to their geographical origin distinguishing two major clusters, one comprising cultivars originated in the Mediterranean countries and the other from Iran and the Caspian Sea. Those studies were continued later (Hormaza et al. 1998), increasing both the number of genotypes closer to the pistachio center of origin and the number of RAPD primers. The results obtained with 29 genotypes and 37 primers agreed with earlier observations since most of the new genotypes fell into the Iranian-Caspian cluster. RAPD markers have also been used more recently to study the diversity of local pistachio germplasm in Turkmenistan (Barazani et al. 2003).

Recently, a first set of microsatellite markers has been developed in pistachio (Ahmad et al. 2003). In this work, a genomic library enriched for dinucleotide and trinucleotide repeats from the cultivar 'Kerman', was used to identify 14 SSRs that resulted in 46 putative alleles in a set of 17 pistachio cultivars (six from Syria, eight from Iran and two from Turkey). These microsatellites have been initially used by Ahmad et al. (2003) to identify the set of cultivars studied, analyze their genetic similarity and to establish a true to type assay based in the DNA extraction from pistachio kernels and shells. Twelve of those markers, together with 104 polymorphic markers produced by eight primer combinations following the Sequence-Related Amplified Polymorphism (SRAP) technique, have also been recently used to identify four commercial pistachio rootstocks (*P. atlantica* cv. 'Standard Atlantica', *P. integerrima* cv. 'Pioneer Gold', and the *P. atlantica* × *P. integerrima* hybrids 'PGII' and 'UCB-1') detecting variation in the UCB-1 rootstock (Ahmad et al. 2005).

At the interespecific level, molecular DNA markers have been used in *Pistacia* to analyze the phylogenetic and similarity relationships among the species of the genus. Thus, Parfitt and Badenes (1997) determined the phylogenetic relations among 10 *Pistacia* species using PCR-RFLP chloroplast DNA analysis. This analysis led to the classification of the *Pistacia* species into two main groups, *Lentiscus* and *Terebinthus,* with all the species of the former group being evergreen with paripinnate leaves, and the species in *Terebinthus* group, including *P. vera*, being deciduous with imparipinnate leaves. This work confirmed the morphological observations of Zohary (1952) that *P. vera* and *P. khinjuk* are the most primitive *Pistacia* species and thus confirming Central Asia, the natural area of this species, as the origin of diversity of the genus. The interspecific relationships described by Parfitt and Badenes (1997) using chloroplast DNA were later confirmed by Kafkas and Perl-Treves (2001) using RAPD nuclear DNA markers. Additionally, Kafkas and Perl-Treves (2001) were able to separate *P. vera* from *P. khinjuk* and established species-specific RAPD markers for the identification of unknown *Pistacia* germplasm. In a subsequent study, Kafkas and Perl-Treves (2002) analyzed the interspecific relations of nine *Pistacia* species using RAPD markers, and included two species (*P. palaestina* and *P. eurycarpa*) that had not been analyzed before. In this study, the species analyzed grouped in two clusters, one comprising single-trunked trees, including *P. vera*, and a second group comprising shrubs or small trees including *P. lentiscus* and *P. terebinthus,* and differing from the classification made by Parfitt and Badenes (1997). However, other works with RAPDs and AFLPs (Katsiotis et al. 2003; Golan-Goldhirsh et al. 2004) grouped the species in agreement to the initial classification, with one group containing evergreen species and including *P. lentiscus*, and a second group containing deciduous species and including *P. vera* and *P. terebinthus*. RAPDs have also been used to study the genetic diversity of *P. lentiscus* populations in Southern Spain and Northern Africa (Werner et al. 2002). The identification of RAPD markers specific to *P. lentiscus* or *P. terebinthus*, has also allowed to identify hybrid genotypes of *Pistacia* × *saportae* Burnat (*P. lentiscus* × *P. terebinthus*), a *P. vera* rootstock, using RAPD marker profiles (Werner et al. 2001). Recently, a set of microsatellite markers developed in mango by Viruel et al. (2005) have also been used to analyze the genetic relationships among *Pistacia* species and among several pistachio cultivars (Viruel and Hormaza, unpublished data). The study of the transferability of SSR markers between the two genera, showed that 44% of the SSRs developed in mango are conserved in the four *Pistacia* species studied (*P. vera*, *P. atlantica*, *P. therebinthus* and *P. lentiscus*) and, thus, they can be added to the set of microsatellite markers available for studies in *Pistacia* species.

12.2.2 Marker-Assisted Selection

The use of molecular markers linked to sex determination in *Pistacia* is one recent application of marker-assisted selection in this species. Pistachio, as well as other species of the genus, is dioecious, and is characterized by a long juvenile period needing five to eight years to reach maturity. Since morphological markers do not allow distinguishing female from male plants prior to flowering, determination of the plant gender at an early vegetative stage would greatly facilitate breeding, selection and management of this species. The first molecular marker linked to sex determination in *P. vera* was identified by Hormaza et al. (1994b) using bulked segregant analysis (BSA). To identify this marker, the DNA of seven male and seven female pistachio trees from two different crosses (Lassen × Peters and Kerman × Peters) were bulked and screened for polymorphisms with 700 RAPD primers. One RAPD marker ($OPO08_{945}$) was found to be present in female genotypes and absent in male genotypes and, thus, linked to the gene(s) controlling sex determination in *P. vera*. Subsequently, this marker has proved to be useful for sex identification in a large number of genotypes (Yakubov et al. 2005). A similar approach was followed by Kafkas et al. (2001) to identify markers linked to sex determination in the wild *Pistacia* species *P. eurycarpa* and *P. atlantica*, used as *P. vera* rootstocks. In this work, two markers linked to *P. eurycarpa* sex determination (one present in the male bulked DNA and the other in the female bulked DNA), and one in *P. atlantica* (present in the female bulked DNA) were found from the screening of 472 RAPD primers. In both works (Hormaza et al. 1994b; Kafkas et al. 2001), hybridization signals of the identified sex-related RAPD markers were found in repetitive sequences and a low frequency of sex related polymorphisms was observed. These results suggest that sex determination in *Pistacia* species must be restricted to a small region of the genome comprising one or few genes, surrounded by repetitive sequences.

12.3 Future Scope of Works

Fruit tree breeding is hampered by factors that make progress slower and more expensive than other crops. The large generation time of fruit trees makes traditional breeding including crossing, evaluation and selection a lengthy process. Additionally, a great amount of space and resources are needed to maintain living trees. Thus, the size of progenies and breeding programs and, consequently, the variability that can be screened is usually limited. Molecular markers tagging single gene traits or quantitative loci allow early seedling screening, reducing space and resources. Therefore, although crossings can only be carried out at maturity, fruit tree breeding can still greatly benefit from molecular genetics. Transformation can also help to make rapid advance in fruit breeding. The introduction of desired characters in elite germplasm eliminates the long time needed to introduce traits of interest by recurrent crossings. Additionally fruit trees are vegetatively propagated and, therefore, the improvement, once it is introduced, can be maintained through clonal propagation. The drawbacks of transformation are that regeneration protocols are still not available for most fruit tree species and, that the evaluation of transgenic fruit trees is still expensive and time consuming (Scorza 2001).

In pistachio, important advances have been obtained using classical breeding and selection approaches. Thus, germplasm has been characterized using phenotypical descriptors and molecular markers, breeding programs based on controlled crosses of selected genotypes are currently underway in different countries, information of heritability of some traits is available and advances in propagation and regeneration have been reported. However, the integration of molecular tools with conventional methods will be a qualitative advance in pistachio breeding programs. Initial molecular works in pistachio have initiated by molecular marker studies to identify germplasm and to study the genetic variability available. Advances have also been made in the identification of molecular markers linked to traits of interest such as sex determination; these markers allow the rapid and early screening of a large number of seedlings. However, for a widespread use of molecular breeding in pistachio, saturated genetic maps should also be built in this species and other genes of interest should be tagged to carry out marker-assisted selection and map-based cloning. Additionally the availability of genetic maps will allow QTL identification and genome selection. Therefore, strong interdisciplinary breeding programs combined with appropriate networks that put together conventional breeding and molecular techniques are highly needed in pistachio to make a qualitative advance similar to that currently occurring in other fruit tree species such as those of the Rosaceae.

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