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Divergence time estimation of Tunisian Pistachio corresponding to the "Mid-Pleistocene transition"

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Abstract

Pistacia vera tree, represents a valuable fruit farming in Tunisia, this type crop has been growing for a long time particularly in arid and semi-arid regions. North African origin (Tunisia) of *Pistacia vera* is, until now, ambiguous. The Bayesian Markov Monte Carlo Chains (MCMC) analysis implemented in BEAST software was conducted to analyze the *trn*L-F marker and estimate the divergence times of pistachio clades. *Pistacia* was estimated to have originated at 37.60 My, given to the Madrean-Tethyan hypothesis. The divergence of Mediterranean species (*P. integerrima*, *P. terebinthus*, *P. atlanctica* and *P. khinjuk*) was happened between 11.99 My and 1.85 My, which represented the periods of formation of the Mediterranean climate. The divergence of the *Pistacia vera* species from its closest ancestor (*P. khinjuk*) was estimated at 1.85 My, this period was characterized by a succession of abrupt climatic changes causing alternating glacial and interglacial periods and leading to the fragmentation of the range of plant species. The *P. vera* (Tunisia) / *P. vera* (foreign) divergence date was estimated at 0.48 My, corresponding to the Middle Pleistocene or the "Mid-Pleistocene transition. The diversification of the Tunisian Pistachio was significantly affected by important climatic changes in the North hemisphere and the formation of the Mediterranean region.

1. INTRODUCTION

Molecular dating consists of coupling a genealogy of genes reconstructed from polymorphism of nucleotide sequences, with a calibration point, in order to estimate an absolute time of divergence between taxa, and to correlate this divergence with a biogeography event.

Geographically, Pistacia species are found in distinctive regions around the globe. The overlapping areas of distribution of P. atlantica, P. vera, P. lentiscus, P. terebinthus, and P. chinensis, in the eastern Mediterranean, central Asia, eastern Asia, and Africa, form a continual distribution belt from the Atlantic Ocean to the Gulf of Thailand and the South and North China Sea (Kozhoridze et al., 2015).

Many propositions were discussed about the origin of Pistacia genus and their distribution. Al-Saghir (2009) suggested that Pistacia originated in the New World (America), migrated eastwards via the boreotropical land bridge, reached Africa in the Eocene (44 My), and emerged in India and Southeast Asia around 5 My. The existence of a connection between the Eurasian and North American continental plates allowed boreotropical taxa to expand their distributions over both continents (Tiffney, 1985; Morley, 2003). Many other authors believed that Pistacia has originated in Central Asia by 80 My (Parfitt and Badenes, 1999; Kafkas and Perl-Treves, 2001). Lately, Xie et al. (2014) proposed that Pistacia was estimated to have originated at 37.60 My, and the divergence of the Pistacia vera species from its closest ancestor P. khinjuk was estimated at 1.82 My.

Chloroplast DNA (cpDNA) is a widely used molecular marker in plant phylogenetics because it is primarily maternally inherited, exists in multiple copies per cell, and contains both coding and non-coding regions that are useful for reconstructing evolutionary relationships and estimating divergence times.

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Historical analyses of plastid genomes have revealed that the genus Pistacia is monophyletic but exhibits a relatively low chloroplast mutation rate, which can limit the resolution of recent divergence events (Parfitt & Badenes, 1997). More recent studies using complete chloroplast genomes and nuclear markers have estimated that the stem node of Pistacia originated during the late Eocene (approximately 30-40 million years ago), while the major diversification within the crown group occurred mainly during the middle Miocene (around 10-20 million years ago), though estimates vary depending on the dataset and calibration method used (Zarei et al., 2022a). In addition, the complete chloroplast genome of Pistacia vera has been assembled and analyzed, providing valuable information for molecular dating and phylogenetic research (Zarei et al., 2022b). cpDNA markers such as trnC-trnD and atpB-rbcL have also been applied to explore genetic diversity and phylogeographic structure among pistachio cultivars (Parfitt & Badenes, 1997; Zarei et al., 2022b).

This study aims to (1) confirm the divergence times between the different species of the genus Pistacia using plastid trnL-trnF DNA sequences, and (2) estimate the divergence time between the Tunisian and foreign pistachio (USA / Palestine).

2. MATERIAL AND METHODS

2.1. Taxon sampling

Field visits were carried out in the central southern traditional areas of pistachio growing in Tunisia (25 accessions from Gafsa and El-Guetar oasis). We also included ten accessions maintained at the Regional Center for Agricultural Research of Sidi-Bouzid. The Tunisian pistachio germplasm used in this study was clearly described in Choulak et al. (2019). Other sequences of the same *trn*L-*trn*F gene from several species of the genus *Pistacia* were exploited in the divergence time tree (Table 1). DNA isolation, PCR reactions, and DNA sequencing were clearly detailed in Choulak et al. (2019).

2.2 Phylogenetic analyses estimation of divergence times

BEAST (version 1.7.4) (Drummond and Rambaut, 2007) software is used to estimate the divergence time between different species. BEAST uses the Bayesian Markov Monte Carlo Chains (MCMC) to reconstruct, from a highly parameterized evolutionary model, both evolutionary parameters and phylogeny. A particularity of this software allows the definition of distribution (according to normal law, normal log, exponential, uniform, random or other) of the calibrations used as priors, and to take into account the uncertainties on these calibration points often resulting from fossil information (Drummond and Rambaut, 2007). This distinctiveness allows the BEAST software to construct rooted trees in the company of a timescale and to study sequences from a genealogical point of view.

Table 1. The different species of the genus *Pistacia* used in molecular dating.

Species	Accession number	Distribution	Reference
P. vera	MK654683-MK654721	Tunisia	Choulak et al., 2019
P. vera (Pell)	KP055540	USA	Weeks et al.,2014
P. vera (Golan)	AY677204	Palestine	Yi et al.,2008
P. vera (Wen)	EF193139	California	Yi et al., 2008
P. mexicana	DQ390472	Mexico	Yi et al., 2008
P. cucphuongensis	KF664211	East Asia	Xie et al., 2014
P. weinmannifolia	DQ390473	East Asia	Yi et al., 2008
P. integerrima	EF193128	North Africa	Yi et al., 2008
P. lentiscus	EF193130	Mediterranean	Yi et al., 2008
P. terebinthus	EF193132	Mediterranean	Yi et al., 2008
P. chinensis	EF193127	East Asia	Yi et al., 2008
P. khinjuk	EF193129	Mediterranean	Yi et al., 2008
P. atlantica	EF193124	Mediterranean	Yi et al., 2008
Cotinus scoggygria Scop.	KF600601-KF600602	China	Wang et. 2014

BEAUti software was used to generate the input files in XML format supported by BEAST.

It permits to specify the various parameters (priors) to be used in the analysis.

In practice, we used the data described by Xie et al. (2014), the divergence time between the genus Pistacia and the genus Cotinus was 37.6 My, as calibration date. Numerous sequences of the same trnL-trnF gene from several species of the genus Pistacia were exploited.

MCMC was performed for 20 million generations with a 10% burn-in. The BEAST scan generates two types of output files. A "tree" file that contains all the trees generated during the "run" and a «trace» file containing the details of the calculations performed by the Tracer software (version 1.7.4; Drummond and Rambaut, 2007).

This file also gives information on the clades divergence times in the form of TMRCA (Time to the Most Recent Common Ancestor), estimated in millions of years (Ma).

Results were analyzed and visualized with Tree Annotator v1.7.2, and FigTree v1.4 (Rambaut, 2009), using the maximum clade credibility tree and mean node heights.

3. RESULTS

The Pistacia / Cotinus divergence date estimated at 37.6 My was used to calibrate the BEAST analyzes for the trnL-trnF marker. The tree showed reason dates of divergence between Pistacia species (Figure) in part, and between Tunisian and foreign Pistacia vera in another part. The divergence of the species Pistacia mexicana from other species was estimated at 18.54 My (95% HPD 16.1 - 20.4; SD = 0.0941) and that between P. weinmanilifolia / P. cucphuongensis and the other species was estimated at 15.78 My (95% HPD 13.9 - 17.4; SD = 0.0609) (Figure). From the obtained tree, we also concluded the divergence date of P. chinensis (5.3 My (95% HPD 4.9 - 6.9; SD = 0.00843)) (Fig. 1).

The divergence of the Pistacia vera species from its closest ancestor was estimated at 1.85 My (95% HPD 1.8-2.7; SD = 0.0591). The time of divergence between Pistacia vera of Tunisian origin and P. vera of American and Asian origin (Middle East) was estimated at 0.48 My (95% HPD 0.56-0.41; SD = 0.00801) (Figure 1).

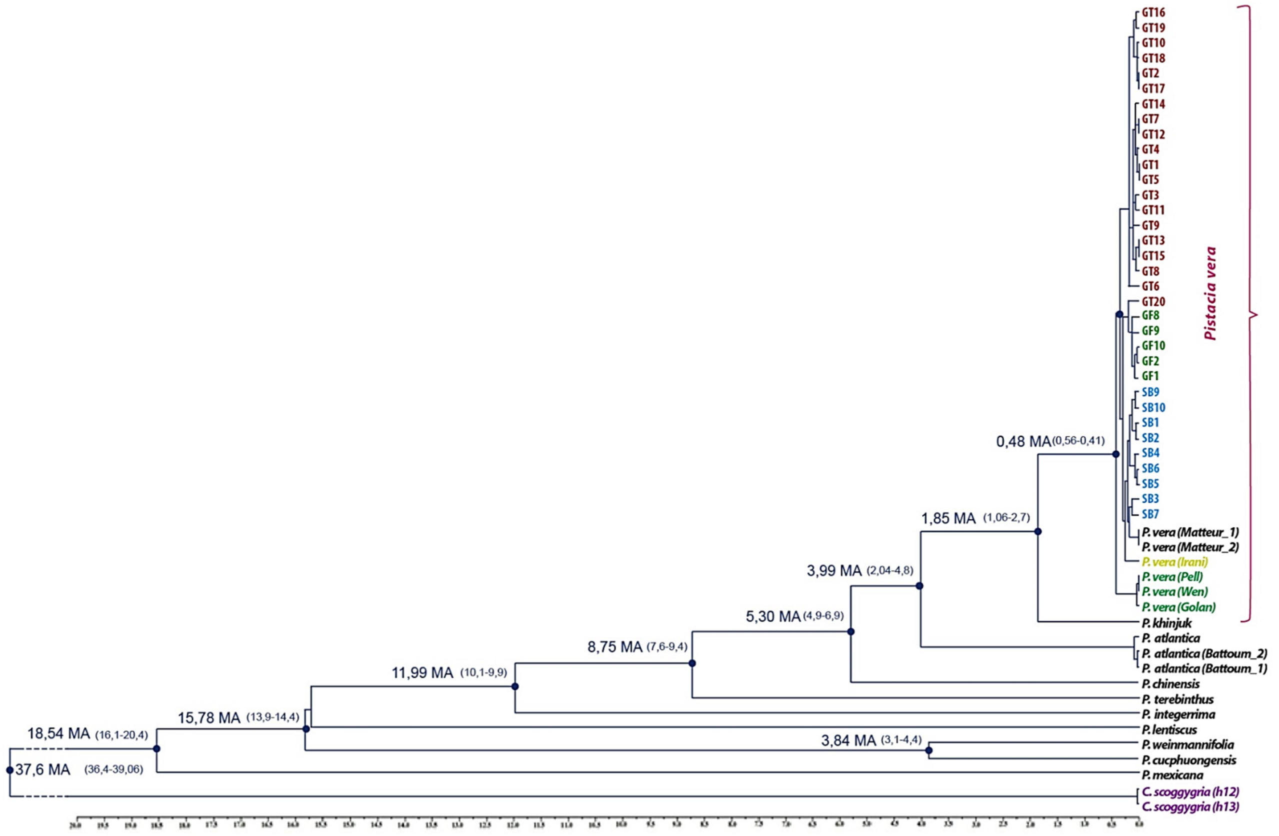

Fig. 1. Estimation of divergence time of genus *Pistacia* based on the combined the *trn*L-F data set using BEAST.

4. DISCUSSION

Molecular dating consists of coupling a genealogy of genes, established from polymorphism of nucleotide sequences, with a well-determined calibration point. The objective is to estimate the time of divergence between taxa and to correlate this divergence with a biogeography event. BEAST software is used to achieve this objective. The estimated Pistacia / Cotinus divergence date of 37.6 My (at the limit of the Oligocene and Eocene) (Xie et al., 2014; Zarei et al., 2022a) was used to calibrate the BEAST analyzes. This divergence date was given to the Madrean-Tethyan hypothesis described by Axelrod in 1975 (Xie et al., 2014). The hypothesis suggests an approximately continuous transatlantic link between the arid flora of western North America, western Eurasia, and North Africa, given the closure of the Mediterranean and the North Atlantic (Axelrod, 1975). These florae were in the form of evergreen sclerophyllous vegetation which underwent an expansion after climatic cooling and aridification at the Eocene-Oligocene limit (Axelrod, 1975). The initial diversification of Pistacia (37.6 My) coincided with this expansion. At least, four species of Pistacia genus (P. lentiscus, P. mexicana, P. texana and P. weimannifolia) still have small, thorny, evergreen sclerophyll leaves, typical of woody evergreen sclerophyll taxa in areas with a Mediterranean climate (Xie et al., 2014). This intercontinental floristic continuity was disturbed at the end of the Oligocene (23 My ago). Floristic migration across the North Atlantic was probably determined through land bridges formed in the Atlantic Ocean (29.72 My - 13.30 My) (Axelrod, 1975; Tiffney, 1985). Indeed, this event was linked to the date of divergence of P. mexicana from the rest of the species of the genus Pistacia (18.54 My). Previous molecular biogeography studies supported this hypothesis. Other taxa of angiosperms presented a similar distribution (Hileman et al., 2001; Feng et al., 2005; Xie et al., 2014).

The divergence between P. weinmannifolia and P. cucphuongensis, native to East Asia, and the other species has been estimated at 15.78 My. This was largely synchronized with the formation of the Tibetan Plateau (Qinghai, Tibet), in Central Asia, beginning at 25-17 My (Guo et al., 2002; Harrison et al., 1992; Spicer et al., 2003). The average altitude of this Plateau exceeds 5000 m. Another dispersal event of Mediterranean regions and East Asia was estimated during the late Miocene. Indeed, the Qinghai-Tibetan Plateau had experienced other significant increases during 10 - 3 My (Harrison et al., 1992). This disjunction was probably the cause of the observed divergence of Pistacia chinensis (East Asia) and the [P. atlantica - P. khinjuk - P. vera] (Mediterranean region) about 5.3 My ago. The elevation of the Qinghai-Tibetan plateau and associated climate change (extreme drought, desertification, and fragmentation of forest communities) constituted a natural barrier for species dispersal (Xie et al., 2014). Several previous molecular studies presented similar results. The disjunction between East Asia and Mediterranean Eurasia has been attributed to divergences between many species and even to the extinction of several Taxa (Sun, 2002; Qiao et al., 2007).

The divergence of Mediterranean species (P. integerrima, P. terebinthus, P. atlanctica and P. khinjuk) was happened between 11.99 My and 1.85 My, period which corresponds to the Miocene, Pliocene, and Pleistocene. The origin of this Mediterranean speciation can be attributed to the period of formation of the Mediterranean climate. The geography of this part of the world changed radically during the Miocene (15 My): a displacement of Africa caused the closure of the sea at the level of the current Middle East and blockage of the Strait of Gibraltar (Thompson, 2005). This progressive isolation of the Mediterranean Sea, from the Atlantic Ocean to the west and the Indian Ocean to the east, produced dramatic changes in terrestrial environments of the Mediterranean region. The Mediterranean was almost completely formed 5 My ago. During the late Miocene, the climate of the Mediterranean region underwent continuous cooling and aridification (Thompson, 2005). These changes can also be at the origin of this diversification. Indeed, the origin of the speciation of several plant Taxa has been dated to the Pliocene, Oligocene, and Miocene, which represented the periods of formation of the Mediterranean climate (Caujapé-Castells et al., 2001; Vargas et al., 2009; Xie et al., 2014).

The divergence of the Pistacia vera species from its closest ancestor (P. khinjuk) was estimated at 1.85 My, which corresponds to the Pleistocene. This was a time of significant global cooling (Sosdian and Rosenthal, 2009). This period was characterized by a succession of abrupt climatic changes causing alternating glacial and interglacial periods and leading to the fragmentation of the range of plant species (Nilsson, 1983).

The P. vera (Tunisia) / P. vera (foreign) divergence date was estimated at 0.48 My, corresponding to the Middle Pleistocene. The cooling that has been evident since the Miocene has intensified with the appearance of the glacial-interglacial climatic cycles of the Northern hemisphere. It was further accentuated with the increase in the duration of these cycles (Maslin and Ridgwell, 2005; Joannin, 2007). According to the authors, this period occurred between 1.2 and 0.5 My (Head & Gibbard, 2005). At this time, global ice volume was increasing. This transition has become known as the 'mid-Pleistocene revolution' or more specifically the 'mid-Pleistocene transition'. The impact of this transition on terrestrial and marine biota has been profound (Head and Gibbard, 2005). This response to changing environments may be the major cause of the observed diversification between native and foreign pistachio.

5. CONCLUSION

The application of the plastid marker, to estimate divergence time of pistachio species, was efficient. The diversification of the genus Pistacia in the Old World was considerably affected by extensive geological and climatic changes and evidently the formation of the Mediterranean region.

The divergence time of the Tunisian pistachio was estimated at 0.48 My, corresponding to the Middle Pleistocene. The climate changes, in this epoch, particularly the increasing severity and duration of cold stages, have had a profound effect on the biota and the physical landscape, especially in the northern hemisphere (Head & Gibbard, 2005).

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