PROPAGATION OF MASTIC TREE: FROM SEED TO TISSUE CULTURE

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Abstract

Mastic tree (Pistacia lentiscus L.*) is a natural element of Mediterranean maquis vegetation. Although, Pistacia lentiscus var. chia is the unique variety from which the gum mastic is economically obtained, only grown in Chios island of Greece. It's a known fact that the mastic tree was also grown on a limited scale in Çeşme Peninsula in the past. Gum mastic is being used in food, cosmetic and pharmaceutical industries in currency. In recent years, it was attempted to restart the growing in Çeşme Peninsula. Mastic tree is a dioecious plant and only male plants are used in mastic production. So the mastic tree doesn't propagated by seed. Mastic tree has been propagated by wood cuttings. Cuttings have been directly sticking into the soil where the plantation be established. Leafy semi-hardwood cuttings have also rooted at high percentages up to 76%. Success in propagation by grafting/budding is time dependent and limited. Air layering is also used in nursery tree production. Micropropagation by in vitro proliferated shoots from mature mother plants was unsuccessful. Although, success rates are quite high in regenaration of explants obtained from in vitro axenic embryos besides in vitro micrografting. Testing the genetic stability and gender in new plantlets obtained are obligatory. As for the micrografting, heterogenity of seedling rootstocks and possible scion/stock interactions have to be considered. In this paper, different propagation methods of mastic tree were exposed and discussed.*

Keywords: *Mastic tree, propagation, cutting, layering, micropropagation*

INTRODUCTION

Mastic tree (*Pistacia lentiscus* L.) is an evergreen shrub or small tree, belonging to Sumac family (*Anacardiaceae*). It is a leading component of Mediterranean maquis vegetation (Zohary, 1995). It is the unique species evaluated for gum mastic. Mastic is a resin obtained from *P. lentiscus* var. *chia* Duham. which is differed from the wild species with some morphological features (Browicz, 1987; Zakynthinos and Rouskas, 1998) The oldest information on mastic goes back to Herodotus (5th century B.C.). In traditional Greek medicine, mastic had been used particularly for gastrointestinal disorders. Ancient Greek and Roman physicians mentioned about the therapeutic properties of mastic and recommended its use (Browicz, 1987; Belles, 2008; Paraschos et al., 2012). Today, mastic resin and mastic oil are the products with high added value and used in many industrial applications (Chadzopulu et al., 2011; Freedman, 2011; Paraschos et al., 2012). In Chios island, about 140t/year mastic is being produced from nearly 2 million trees in currency. Figures of mastic production and the number trees did not change for a long time (Belles, 2008). Mastic tree is a dioecious tree and only male trees have been used through the centuries because of their high quality resin. So, mastic tree is propagated clonally by the hardwood cuttings (Baytop, 1968; Browicz, 1987; Belles 2008, Zografou et al., 2010). Despite the mastic tree and mastic gum have been associated with Chios through the history, mastic tree cultivation had been also conducted in the adjacent Western Anatolia in the past (Baytop, 1968; İsfendiyaroğlu, 2003). Remnants of old plantations on Çeşme Peninsula pointed out the former growing activities on a limited scale, but gradually lost its importance in time. In recent years, some attempts were started aiming on the protection and rehabilitation of the current genetic material, by some governmental institutions and NGOs. Moreover, establishing the new plantations and restart the mastic production in alternative lands are encouraged. Recent planting activities in Turkey seemed to be increased the demand to nursery tree production in the coming years. So, prediction of the most proper propagation method is of great importance. In this review, current and potential propagation methods of mastic tree were presented and discussed.

Propagation of Mastic Tree

Seed Propagation: Mastic tree is a dioecious plant and pollinated by wind (Zohary, 1952). In *Pistacia* species, viable pollens drift as much as half a mile or sometimes more, cause seedling diversity (Joley and Opitz, 1971). Gum mastic is being obtained only from male trees (Browicz, 1987; Belles, 2008). There are several cultivated male clones differing in quality and quantity of mastic resin that show genetic diversity (Browicz, 1987; Belles, 2008; Zografou et al., 2010). So, the seeds should not be used to propagate the mastic tree, because of genetic variability. Seed propagation can be used in reforestation and environmental rehabilitation programmes (Piotto and Di Noi, 2003). And it is also considered in breeding works and evaluating as ornamental nursery tree. Marked differences were pointed out among the *P. lentiscus* genotypes, related with the extend of fruiting, percentage of aborted ovaries, viability of seeds, percentage and speed of germination (Grundwag, 1975; Mulas et al., 1998; Piotto et al., 2003; Fascella et al., 2004). The low seed setting in *Pistacia* species is attributed mainly to degeneration of female reproductive structures (Grundwag, 1975; 1976). In mastic tree, fruit colour is strongly associated with the seed viability and black fruits usually contain viable seeds, whereas the red ones contain non-viable seeds in relation with parthenocarpy or ovary abortion (Garcia-Fayos and Verdu, 1998). Seeds of *P. lentiscus* do not show dormancy. But the seeds are rich in oily substances, therefore the seed viability could be lost in a comperatively short course of time (Garcia-Fayos and Verdu, 1998; Piotto et al., 2003). In fact, seeds showed a significant and strong decrease in germinability over time (6 months) under standard storage conditions (21ºC and 6% moisture content) (Garcia-Fayos and Verdu, 1998). Pulp removal is a necessary treatment for germination success (Piotto, 1995; Garcia-Fayos and Verdu, 1998; Piotto et al., 2003). Mastic tree seeds are quite small (1000 seeds = 10-25g), so it is impossible to scarify them by hand. Short durations of cold stratification (3-4 weeks at +3-4°C) seemed to be sufficient for completing the dormancy removal in seeds (Piotto, 1995; Piotto et al.,2003; Fascella et al., 2004). Increases in time of cold stratification generally resulted with the decreases in percent germination of seeds (Garcia-Fayos and Verdu, 1998; Karakır and İsfendiyaroğlu, 1999; Fascella et al., 2004). Combined effects of scarification (50 sec) with cold stratification (7 and 21 days) did not significantly affect the percent germination of *P. lentiscus* seeds, and germination rates ranged between 75 and 81% (Piotto, 1995). The effects of acid (1NHCl for 20 min) scarification or soaking with GA_3 (1000 ppm for 20 min) after cold stratification seemed to be partially genotype dependent, but generally increased the germination of seeds compared to untreated ones (Fascella et al., 2004). GA_3 applications (24 h) ranged between 0 and 200 ppm did not significantly change the germination of mastic tree seeds that scarified with H_2SO_4 (10 min) and stratified at +2°C, throughout 60 days (Karakır and İsfendiyaroğlu, 1999). In case of seed propagation, mature black fruits have to be collected in October-November and subsequently subjected to mesocarp removal. Scarification is useful to speed up germination and homogenize the seedling emergence. Alternatively, cold stratification may be applied. Sowing in autumn, without any pretreatment or spring sowing of either scarified or cold stratified (2-3 weeks) seeds were recommended (Piotto et al., 2003; Prada and Arizpe, 2008). Mean height and stem diameter were measured as 12.5 cm and 2 mm respectively in 11 month old seedlings were grown under controlled conditions (Karakır and İsfendiyaroğlu, 1999).

Cutting Propagation: Mastic tree has been propagated clonally by the hardwood cuttings through the history. Thick branches are collected from the mature trees and planted at their final places where the tree is grown. The planting holes must be dug one year earlier, at a depth of 70-80 cm and let to aerated. Cuttings are planted in a depth of 40-50 cm and a little sideling. The planting time is December so that the highest use of precipitation. During the first summer after planting, cuttings are regularly watered and fertilized (Browicz, 1987; Belles, 2008). Wood cuttings are also rooted in containers filled with good aerated soil based mixtures in lath house or in simple greenhouses. Leafy cuttings from mature trees of *Pistacia* species are considered very difficult-to-root (Joley and Opitz, 1971). Cutting collection time is crucial for rooting success and cuttings could be rooted in a very short period of the year. In this period, the effect of applied auxins is more pronounced (Avanzato and Damiano, 1990; Dunn et al., 1996b). Rooting of *Pistacia* cuttings from mature stock plants is limited even with the high auxin concentrations and auxin combinations (Al Barazi and Schwabe, 1982; Dunn et al., 1996a). Ontogenetic juvenility in *Pistacia* species seems to be lost after a few years generally, making the rooting of cuttings more difficult (Lee et al., 1976; Al Barazi and Schwabe, 1982; Pair and Khatamian, 1983). Semi-hardwood cuttings of mastic tree showed a short period of rootability (İsfendiyaroğlu, 2000; Viola et al., 2004; Pignatti and Crobeddu, 2005). Even the effects of exogenously applied auxins and their combinations are more pronounced in proper time of collection (İsfendiyaroğlu, 2000). In leafy semi-hardwood cuttings from mature *P. lentiscus* var. *chia* trees, the highest rooting (76.6%) was obtained when they were collected in mid-February and treated with 20 $g.l^{-1}$ IBA as quick-dip, in pure perlite (coarse), with bottom-heating (25°C), under mist. In the second year, mid-February cuttings also gave the highest percent rooting 43.3% with IBA-NAA mix at 10+2.5 $g.l^{-1}$. Lower rooting rates were obtained with IBA treatments up to 40 $g.l^{-1}$ (İsfendiyaroğlu, 2000; 2003). Micro-anatomical observations showed that, mastic tree cuttings showed indirect rhizogenesis and root primordia originated from basal callus tissue. The emergence of adventitious roots was observed after 45th day or onward (Karakır and İsfendiyaroğlu, 1999). In leafy semi-hardwood cuttings of selected mastic tree clones, February seems to be the most proper collection period. IBA and its mixtures with low amounts of NAA as quick-dip is necessary to obtain the highest number of rooted cuttings. The rooted cuttings showed a rapid shoot growth after transplanting to the soil based growing medium and reached up to 50 cm high at the end of the active growing season (Karakır and İsfendiyaroğlu, 1999).

Propagation by Layering: Air layering seems to be an alternative method to propagate mastic tree when the limited quantity of nursery tree is needed. It was basically an ancient method used to propagate a number of tropical and subtropical trees and shrubs. Today, this method is useful for producing a few plants of relatively large size for special purposes (Nelson, 1987; Hartmann et al., 2002). In mastic tree, branches that more than 1.5 cm in diameter found to be most suitable for layering. Girdling the stem by removing a strip of bark 0.5-1 cm wide completely around the stem is necessary. A 15x25 cm black polybag passes from the distal end of the branch up to girdled area, after evenly cutting its closed bottom edge. The lower end of the bag should be twisted and filled with about 1 l of moist peat. After then, both ends of polybag are wrapped with electrical tape. Making layering in mid-summer and removal of rooted branches from the mother plant at the beginning of spring are recommended. Rooted layers are transplanted in 4 l containers after a slight pruning on the top. In this method, success is ranged between 85-90%, but the number of layers is closely related with the canopy size of the mother plant used. Excessive application of layers could be detrimental for mother plants was suggested (Tutar et al., 2016b).

Propagation by Budding/Grafting: In the history of mastic culture, clonal propagation by budding/grafting did not take place in practice. However, it is possible to meet a few examples of grafted trees scattered around the Çeşme peninsula (Western Turkey). In recent years, some experimental works conducted on prediction of proper method of budding/grafting onto other *Pistacia* rootstocks (Tutar et al., 2016a; Parlak, 2018). Among the five different *Pistacia* seedling rootstocks assessed, the highest bud-take (45.3%) was obtained from *P. atlantica* with "T" budding was done at the end of summer (Tutar et al., 2016a). From mid-February to mid-March was the best period for chip budding and cleft grafting onto *P. atlantica* and *P. lentiscus* seedlings, under controlled conditions was reported. And the highest graft-take (47.8%) was observed in cleft grafts of mastic tree/*P. atlantica* combination carried out in 15 February (Parlak, 2018). *P. atlantica* seedlings seem to be the most proper rootstock for the clonal propagation of mastic tree, because of its high vigor. However, mastic resin and essential oil yields did not measured in the mentioned rootstock studies. Moreover, chemical composition of essential oils from budded/grafted trees should be clarified, because of the probable scion/stock interactions (Hartmann et al., 2002; Mudge et al., 2009). In fact, inconsistent figures were obtained in relation with the essential oil content of mastic resins, when the self- rooted trees compared with the trees that were budded onto different rootstocks (Tutar et al., 2016a).

Micropropagation: Early works showed that, browning of plant explants caused by phenolic exudation was the major constrain in micropropagation of *P. lentiscus* (Fascella et al., 2004; Ruffoni et al., 2004; Mascarello et al., 2007). Propagation from axenic seeds of female mastic trees was proposed as a novel protocol to obtain nursery trees in large quantities (Yıldırım, 2012; Kılınç et al., 2015; Koç et al., 2014). Although the genetic stability of regenerated plants were studied in terms of polymorphism (Kılınç et al., 2015), gender of the new plantlets remain obscure. Root regeneration of *in vitro* proliferated shoots from mature mother plants of mastic tree was unsuccessful. So, micrografting of mastic tree onto seedling rootstocks of different *Pistacia* species was developed (Onay et al., 2016). In this method, shoots (one-year-old wood) from mature mastic trees were collected in mid-May and forced to sprout axillary buds in solid (peat, perlite) and liquid media *in vivo*. And shoot tips were proliferated by subcultures for using as a source of scion. The highest grafting success (100%) was recorded from *in vitro* regenerates from both male and female genotypes. Despite the high frequency of regeneration was achieved by this protocol, low frequency of genotypic variation in regenerated plants has to be considered. Moreover, all the *Pistacia* species used as seedling rootstock in this study were deciduous by contrast with mastic tree, and possible disorders could be derived from scion/stock interactions (Simons, 1987; Garner, 2007; Hartmann et al., 2002; Mudge et al., 2009) were ignored in this protocol.

In conclusion, among the different methods assessed, propagation by leafy semi-hardwood cuttings seems to be the most proper method in mass production of mastic tree clones. Despite the success of rooting depends on a limited cutting collection time, rooting rate of cuttings were at the commercially acceptable levels, even the mother plants were in mature phase. Rejuvenation of mother plants seems to be crucial for the high root regeneration in cuttings. Determination of auxin sensitive phase of rooting in cuttings should be determined for high efficiency of applied auxins and their combinations. Further nutrition programs can be developed for rapid growth of rooted propagules.

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