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Effects Of Some Physical And Biochemical Factors On The Rooting Of Mastic Tree (*Pistacia lentiscus* var. *chia* Duham.) Cuttings

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Özet

Bazı Fiziksel ve Biyokimyasal Faktörlerin Sakız Ağacı (*Pistacia lentiscus* var. *chia* Duham.) Çeliklerinin Köklenmesi Üzerine Etkileri

Bu çalışmada sakız ağacının, yapraklı uç çeliklerinin sisleme altında köklenmesi üzerine farklı çelik alma zamanları, köklendirme ortamları ve indolbutirik asit (IBA) konsantrasyonlarının etkisi incelenmiştir. Ayrıca, çeliklerin içsel köklenme kofaktörü aktivitesi belirlenmiştir. En yüksek köklenme oranı (% 76.6), 15 Şubat'ta alınan ve 20 $g.l^{-1}$ IBA uygulanan çeliklerde, perlit ortamında elde edilmiştir. Çeliklerin köklenme oranlarındaki azalmalara karşın, engelleyici köklenme faktörlerinin (Rf 0.1, 0.9 ve 1.0) sayısı ve mung fasulyesi testindeki aktiviteleri düzenli olarak artış göstermiştir. Elde edilen sonuçlar, sakız ağacının yapraklı odun çelikleri ile kitlesel olarak çoğaltılabileceğini göstermektedir.

Anahtar Sözcükler: Sakız ağacı, *Pistacia lentiscus,* çelik, IBA, köklenme kofaktörü.

Introduction

Mastic tree (*Pistacia lentiscus* var. *chia* Duham.), is the unique source of commercial gum mastic, obtained by wounding the trunk and thick branches of the plant (1, 2). Only male trees are used for mastic production because of the low productivity of female ones (2). Mastic is a natural resin which is presently being used in over sixty areas, mainly in food, perfume and medicine industry (1, 3). It has been associated for a long time with the Greek island of Chios. Although it is not extensive and in quantity as on Chios, mastic had also been produced in the adjacent Western Anatolia, on Çeşme peninsula of İzmir, where the ecological conditions are identical (1, 2). However, mastic tree cultivation was negatively influenced as a result of

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diminishing agricultural activities in the last 20 years in the peninsula. Mastic tree has to be considered as an important loss from plant genetic resources of Turkey. Because, recent field observations indicated that there are a very few trees in the vicinity of Çeşme.

Consequently, clonally propagated nursery stocks would be needed to establish new plantations.

Difficulty in propagation seems to be a limiting factor to extend the cultivation. Traditional propagation is based on direct sticking of hardwood cuttings to the soil where the plantation to be established (2, 3). In this method, rooting success is low and accidental. Budding and grafting were reported to be unsuccessful so far (2). Leafy cuttings of mature mastic tree were poorly rooted with the treatment of 15.000 ppm IBA when taken in March. Subsequent softwood cuttings failed to root (4). Cuttings from mature trees of other *Pistacia* species are considered very difficult-to-root. The most important factor affecting the rootability of *Pistacia* spp. cuttings is the proper cutting collection timing. Cuttings could be rooted, if they were prepared from actively growing shoots (5, 6, 7). Rooting potential of *Pistacia* spp. cuttings significantly decreased in parallel with ontogenetic aging was suggested (5, 8). In this case, softwood cuttings from mature trees were only rooted with the treatments of high IBA concentrations (7, 9). On the other hand, *P. chinensis* cuttings rooted better in solid media had high aerating capacity (7, 10).

Biochemistry of adventitious root formation in cuttings was not entirely cleared out yet. However, easily rooted plants were found to have some rooting cofactors in higher content than the difficult-to-root ones. These cofactors were identified as phenolic compounds and appeared to act sinergistically with endogenous auxin (11, 12).

The objectives of this study are to predict the most proper cutting collection time, rooting medium and IBA concentration to expose the possibilities of cutting propagation of mastic tree. Besides, changes in cofactor activity of the cuttings according to cutting time were examined.

Material and Methods

In this study, five old male trees from the vicinity of Çeşme were selected as stock plants. Leafy hardwood and semi-hardwood tip cuttings were collected at 11 different dates through the experiments (15.Nov.1995-20.Nov.1996). Cuttings were prepared according to İsfendiyaroğlu, 1994 (4) and they were dipped 5 sec. in 10, 15 and 20 $g.l^{-1}$ IBA dissolved in 50 % isopropanol. Cuttings were rooted in a low tunnel misting system. Coarse perlite and 1 peat:1 perlite (v/v) were used as rooting media. Misting frequency was controlled by electronic leaf and bottom heat was adjusted to 25 °C. Cuttings were evaluated approx. 12 weeks after planting for percent rooted cuttings, number of roots and root length per rooted cutting. A randomized block design was used with 3 replications of 10 cuttings for each treatment combination. Statistical analysis were carried out using SPSS 5.0. Data were analysed by ANOVA and Duncan's multiple range test was used to discern differences at the 5 % level of significance.

The plant samples were collected from the 1 cm basal portions (wood+bark) of cuttings in order to determine the cofactor activity. The cofactor (biological) activity in cuttings was determined by the mung bean bioassay (11). *Vigna sinensis* L. was used as the test plant.

Results and Discussion

The analysis of variance indicated the significant differences among the cutting dates in relation to rooting. Most rooted cuttings were observed on 15 Feb. Rooting percentage gradually decreased until 7 Apr. Percent rooting of fall cuttings were significantly low. Cuttings did produce most and longest roots at the dates that the highest rooting percentages occurred (Table 1).

Table 1. Effect of cutting date, IBA concentration and rooting medium on the rooting percentage, root number and root length of mastic tree cuttings.

Date	Rooting percentage (%)	Root number	Root length (mm)
15/11	3.75 e*	0.46 b	18.67 cd
15/12	2.91 e	0.37 b	10.62 d
15/1	14.58 cd	1.08 b	59.12 ab
15/2	45.41 a	2.78 a	81.22 a
2/3	28.75 b	2.96 a	80.99 a
15/3	9.58 de	1.39 b	37.17 b-d
7/4	18.75 c	2.83 a	58.55 ab
5/9	7.08 de	1.25 b	47.53 bc
20/9	3.75 e	0.92 b	29.48 b-d
20/10	7.50 de	1.24 b	53.24 ab
20/11	2.08 e	0.58 b	18.66 cd
Medium			
Perlite	15.50 a	1.86 a	50.97 a
Peat-perlite	10.70 b	1.02 b	39.08 b
IBA($g.l^{-1}$)			
0	6.81 b	0.78 b	43.06 ns
10	12.72 ab	1.57 a	40.05
15	15.90 a	1.67 a	44.13
20	16.97 a	1.74 a	52.86

* Mean separation by Duncan's multiple range test, 5%; ns: non significant.

Only softwood cuttings taken in March and June from actively growing shoots could be rooted in *P.chinensis* and *P.atlantica* (5, 6, 7). Despite only hardwood and semi-hardwood cuttings were used through the experiments, mastic tree had also high rootability in a very limited period as reported in relative species. Rooting media significantly affected the rooting. Perlite gave the higher rooting values than peat-perlite (Table 1). Root cuttings of *P. chinensis* showed the highest number of roots were regenerated in media with 30-40 % air filled porosity (10). Lower root quality of the mastic tree cuttings in peat-perlite medium may be related with the lower aerating capacity of peat, as it was previously reported for a range of plant cuttings (13). IBA treatments significantly affected the rooting of the cuttings. Treatments provided at least twofold increase in percent rooting and root number compared to untreated cuttings (Table 1). Softwood cuttings from mature *P. vera* had highest rooting with 35.000 ppm IBA, and higher concentration was reduced the rooting (9). Since no significant difference was found between the concentrations of 15 and 20 $g.l^{-1}$, it was thought that high IBA concentrations would be necessary for successful rooting even in the mastic tree cuttings.

There was a significant interaction between cutting date and rooting medium. Cuttings taken on 15 Feb. gave the highest rooting percentage in perlite but significantly lower value in peat-perlite. The highest number of roots were on 2 Mar. followed by 15 Feb. in perlite. The longest roots were from cuttings taken on 15 Feb. and 2 Mar. successively in peat-perlite. A sharp decrease in rooting values was observed on 15 Mar. But on 7 Apr., rooting values were significantly high in peat-perlite medium (data were not shown).

The results brought to mind that peat-perlite was improper for good root quality in the mastic tree cuttings. Relatively high values of 7 Apr. cuttings in peat-perlite may have concerned with the high water holding capacity of the peat material. It was reported that water loss of the cuttings was lower in peat-perlite mixtures in spring, when the air temperature and irradiance became increased (14).

Significant interactions were predicted between cutting date and IBA concentration, rooting medium and IBA concentration in relation to percent rooting and root number. Cuttings that were taken on 15 Feb. mostly responded to increasing levels of IBA and the highest rooting percentage was obtained with 20 $g.l^{-1}$. Rooting percentages were sharply decreased on 2 Mar. in spite of IBA treatments. Following dates, cuttings did not respond to increasing levels of IBA. Also, the effects of IBA applications were more pronounced in perlite (data were not shown). Root number of the cuttings even constantly augmented in parallel to IBA treatments on 15 Feb. But the highest value was obtained on 2 Mar. with 15 $g.l^{-1}$, and increase in concentration reduced the root production (Table 2). Mature *P. chinensis* had very short period (4 weeks) of rootability. And rooting was highest only in softwood cuttings taken on May with 8.750 $mg.l^{-1}$ IBA, two or fourfold increase in concentration reduced the rooting. Nevertheless, the delay of 15 days in cutting date was significantly decreased the percent rooting and root numbers, but relative effects of IBA treatments did not change (7). Similar results were even reported for root numbers of *P. atlantica* softwood cuttings (6). Mastic tree cuttings had also a time depending response to applied IBA as formerly reported in other *Pistacia* spp. On the other hand, cuttings responded well to IBA concentrations up to 20 $g.l^{-1}$ on 15 Feb. (Table 2).

In this respect, higher concentrations may be useful to increase the percent rooting, regardless of root quality (number, length, thickness etc.).

Table 2. Interactions of cutting date and IBA concentration, rooting medium and IBA concentration on the root number of mastic tree cuttings.

	Root number IBA ($g.l^{-1}$)			
Date	0	10	15	20
15/11	1.00 e-g	0.00 h	0.00 h	0.83 e-h
15/12	0.00 h	1.00 e-h	0.00 h	0.47 f-h
15/1	0.33 gh	1.08 e-h	1.62 c-h	1.28 d-h
15/2	1.75 c-h	2.73 b-f	3.00 a-e	3.62 a-c
2/3	0.50 f-h	2.88 a-e	5.00 a	3.45 a-d
15/3	0.50 f-h	1.55 c-h	1.83 c-h	1.83 c-h
7/4	1.25 d-h	4.38 ab	3.12 a-e	2.58 a-e
5/9	0.83 e-h	0.25 gh	1.75 c-h	2.17 c-h
20/9	0.50 f-h	1.50 c-h	1.17 d-h	0.50 f-h
20/10	1.13 e-h	1.42 c-h	0.83 e-h	1.58 c-h
20/11	0.83 e-h	0.50 f-h	0.17 h	0.83 e-h
Perlite	0.87 c	2.36 a	1.92 ab	2.30 a
Peat-perlite	0.70 c	0.78 c	1.41 a-c	1.18 bc

* Mean separation by Duncan's multiple range test, 5%.

Interactions between cutting date, medium and IBA concentration did not significantly affect the rooting of cuttings. However, marked differences have seemed among the rooting percentages. Cuttings taken on 15 Feb. and treated with 20 $g.l^{-1}$ IBA in perlite had mostly (76.6 %) rooted (Table 3).

From this point of view, mid-Feb. appears to be the best time to collect cuttings in Çeşme conditions. Concentration of 20.g.l^{-1} IBA and coarse perlite or media which have similar physical features can be recommended for successful rooting.

Table 3. Interactions of cutting date, rooting medium and IBA concentration on the rooting percentage of mastic tree cuttings.

	Medium							
	Perlite				Perlite-peat			
	IBA(g.l^{-1})							
Date	0	10	15	20	0	10	15	20
15/11	10.00	0.00	0.00	10.00	10.00	0.00	0.00	0.00
15/12	0.00	0.67	0.00	16.67	0.00	0.00	0.00	0.00
15/1	6.67	20.00	36.67	3.33	0.00	0.00	10.00	3.33
15/2	13.33	60.00	66.67	76.67	20.00	20.00	53.33	53.33
2/3	0.00	36.67	43.33	33.33	10.00	33.33	26.67	46.67
15/3	6.67	26.67	16.67	13.33	3.33	0.00	10.00	0.00
7/4	3.33	20.00	16.67	3.33	20.00	30.00	30.00	26.67
5/9	13.33	6.67	6.67	6.67	0.00	0.00	10.00	13.33
20/9	3.33	3.33	0.00	0.00	3.33	0.00	6.67	13.33
20/10	13.33	10.00	10.00	13.33	6.67	0.00	3.33	3.33
20/11	3.33	6.67	3.33	0.00	0.00	0.00	0.00	0.00

In order to determine the cofactor activity, cutting samples belonging to three different dates (15 Feb., 2 Mar. and 7 Apr.), when great differences occurred in rooting percentages of the cuttings (Table 1), were examined. Rooting factors represented as Rf values significantly ($p<0.01$) affected the the rooting of *V. sinensis* hypocotyl cuttings. As seen from histograms (Figure 1), rooting inhibition only occurred at Rf 1.0 on 15 Feb., since the other Rf bands showed the cofactor activity and promoted the rooting. On 2 Mar., when the rooting rates of the mastic tree cuttings were significantly declined, inhibiting effect also occurred at 0.9 in addition to Rf 1.0. But inhibition became more pronounced at Rf 0.9 on 7 Apr. Moreover, a slight activity was also observed at Rf 0.1.

This suggests a possible relationship between in vivo rooting of the cuttings and activity of rooting factors. As a matter of fact, number of inhibitory Rf bands and inhibiting activity at Rf 0.9 constantly increased at the dates that the cuttings tended to loose their rootability. Similarly, cutting extracts from easy and difficult to root forms of *Hibiscus rosa-sinensis* were found to be inhibitory at Rf 0.1, 0.9 and 1.0 (11). Extracts of highly rooted ringed cuttings of *Anacardium* *occidentale* (cashew) showed promotory effect at all Rf values while extract from non-ringed cuttings did inhibit the rooting of bean cuttings at Rf 0.1, 0.5 and 0.7 (15).

Although some differences seem among plant species and treatments, inhibitory factors at Rf 0.1, 0.9 and 1.0 apparently have important roles on the rooting of mastic tree cuttings.

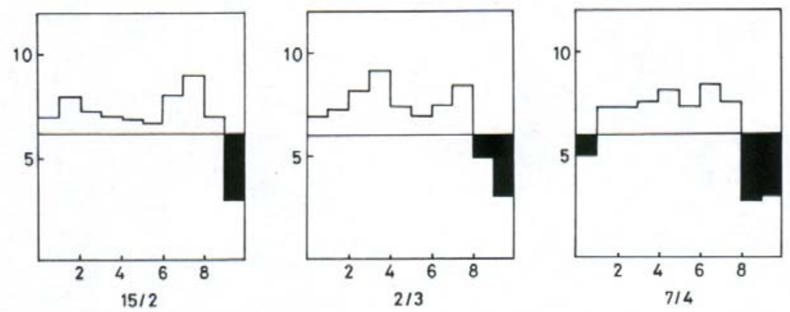

Figure 1. Histograms show the cofactor (biological) activity. Ordinate: average number of roots per mung bean hypocotyl. Abcissa: Rf values in isopropanol: water (8:2 by volume).

Conclusion

Mastic tree cuttings seemed to have a limited period of high rootability. But considering the proper cutting collection time, rooting medium and IBA concentration, this plant can be propagated in large quantities. Also, new studies should be designed on the basis of prediction of new rooting media and the concentration of auxin and/or auxin combinations as a first step to optimize the propagation by cuttings.

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Summary

Effects Of Some Physical And Biochemical Factors On The Rooting Of Mastic Tree (*Pistacia lentiscus* var. *chia* Duham.) Cuttings

In this study, the effect of different cutting collection times, rooting media and indole butyric acid (IBA) concentrations on the rooting of mastic tree leafy tip cuttings, under mist were

examined. Besides, endogenous rooting cofactor activity of the cuttings were predicted. The highest rooting percentage (76.6 %) was obtained from the cuttings taken on 15 February and treated with 20 $g.l^{-1}$ IBA, in perlite. Number of inhibitory rooting factors (Rf 0.1, 0.9 and 1.0) and their activities in mung bean bioassay, constantly increased in contrast to diminishing rooting rates of cuttings. The results indicate that the mastic tree can be propagated by leafy -hardwood cuttings in large quantities.
Key Words: Mastic tree, *Pistacia lentiscus,* cutting, IBA, rooting cofactors.

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