

## AN EFFECTIVE PROTOCOL FOR IN VITRO GERMINATION AND SEEDLING DEVELOPMENT OF LENTISK (*Pistacia lentiscus* L.)

Hakan Yıldırım<sup>1✉</sup>, Nazan Çalar<sup>2</sup>, Ahmet Onay<sup>2</sup>

<sup>1</sup> Malatya Turgut Ozal University, Faculty of Agriculture, Department of Horticulture, 44 000 Malatya, Turkey

<sup>2</sup> Dicle University, Faculty of Science, Department of Biology, 21280 Diyarbakir, Turkey

### ABSTRACT

Different nutrient media (MS [Murashige and Skoog 1962]; QL [Quoirin and Lepoivre 1977] and WPM [Lloyd and McCown 1980]); plant growth regulators BA (benzil adenin), GA<sub>3</sub> (gibberellic acid), IBA (indole-3-butyric-acid), NAA (naftalen acedic acid); and sucrose concentrations were studied to determine the *in vitro* culture effects on healthier and faster seedling development from mature lentisk (*Pistacia lentiscus* L.) seeds. After 28 days of culture, the percentage of germinated seeds was the highest (70%) in the full-strength MS medium. The cytokinin BA was superior to other tested treatments in terms of its ability to promote germination of lentisk seeds. When tested at different concentrations, sucrose gave the best results obtained at concentrations of 1–4%, whereas high concentrations (6 and 8%) mainly decreased germination rate and there was no a regular pattern for elongation of the aerial parts of plants. With this described protocol, on average 76.67% seeds germinated 4 weeks after culture. Developed seedlings were satisfactorily acclimatized in sterilized peat, soil and perlite containing compost, with high percentage survival viability was obtained 9 months after transfer to *in vivo* conditions (93.33%). The results obtained showed that the enriched full-strength MS medium supplemented with 1 mg L<sup>-1</sup> BA and 3% sucrose induced homogeneous and healthy seedling development in a period of 4 to 8 weeks of culture.

**Key words:** mastic tree, micropropagation, seed

### INTRODUCTION

Mastic tree, also known as lentisk (*Pistacia lentiscus* L.), is a dioecious shrub, a member of the Anacardiaceae family, grown commonly in Mediterranean sclerophyllous scrublands from Maghreb to Greece and Turkey [Zohary 1952]. Mastic Gum is stimulated by the incision of the trunk and of the branches of Mastic tree in the form of long, ovoid, pale yellow, brittle tears. It is a source of sustenance for a multipurpose use. It was also reported that *P. lentiscus* like all of the other *Pistacia* species and

their hybrids may be used as rootstock for the establishment of pistachio orchards [Özbek and Ayfer 1959]. Today, production of mastic gum is located only in the traditional province of the Greek Island of Chios. Currently, *P. lentiscus* is naturally propagated by seed with not only consequent increase of genetic variability but also high differences on germination rate among genotypes [Mulas et al. 1998] due to the problems such as inadequate pollination and ovary abortion [Grundwag 1976]. Germination of *P. lentis-*

✉ hakan.yildirim@inonu.edu.tr, hakanyildirim75@hotmail.com

*cus* seeds also decreases with the time of storage [Mulas et al. 1998]. These characteristics of the seeds reduce the rate of natural propagation. Moreover, cuttings from mature trees of *Pistacia* species are also considered to be very difficult-to-root [Karakır and İsfendiyaroğlu 1999]. In the Aegean coastal areas of Turkey, long-term land use and the development of tourism industry has frequently resulted in the disappearance of lentisk. In the degraded coastal areas of Çeşme peninsula (İzmir), an increase in anthropic pressure may be decreased spontaneous colonization, and species reintroduction may be an essential step in the restoration of these habitats. *P. lentiscus* is also important for ecosystem functioning because they protect the soil from erosion, recover quickly after disturbance, and promote biodiversity by facilitating other vascular plants, thus providing shelter and food for fauna [Maestre and Cortina 2005]. Recently demonstration orchards were set up in different regions of Çeşme peninsula with the aim of promoting this crop because lentisk may be economically important species in the western coastal areas of Turkey. *P. lentiscus* var. Chios is the leading cultivar of mastic tree and there is an interest in its agronomic improvement. Several authors have investigated ways to overcome problems encountered with conventional propagation (grafting and cuttings) by focusing on developing more suitable propagation techniques [Fascella et al. 2004, Ruffoni et al. 2004, Taşkın and İnal 2005, Mascarello et al. 2007]. High quality plant production techniques must be implemented to ensure the successful extension of this species. Micropropagation procedures for juvenile material established from shoot tips of *P. lentiscus* seedlings have already been established [Yıldırım 2012, Koç et al. 2014]. A regeneration protocol from even juvenile material would allow the possibility for genetic transformation; therefore a protocol to germinate *P. lentiscus* seed *in vitro* would be necessary. However, germination of *P. lentiscus* seeds like another species of the genus *Pistacia* are generally low. Studies on seed germination [Piotto 1995, Mulas et al. 1998] and *in vitro* culture [Fascella et al. 2004, Ruffoni et al. 2004] of these species are very scarce. For this reason, some treatments to improve germination are applied to the seeds before sowing. De-

hulling or splitting, soaking in water, different scarification methods, treating with GA<sub>3</sub> and stratification increase germination rate of *Pistacia* seeds [Ayfer and Serr 1961, Crane and Forde 1974, Kaşka et al. 1992]. Dormancy is one of the efficient factors on seed germination. Seed dormancy is divided into two types: coat-imposed dormancy and embryo dormancy by some researches. Coat-imposed dormancy is derived from the tissues enclosing the embryo (endosperm, pericarp, basically or extrafloral organs). In contrast, the other type of dormancy (embryo dormancy) is derived from the embryo itself [Bewley and Black 1994].

An investigation was carried out to study the germination percentage of seeds and relations between phenolics and seed dormancy of *Pistacia* spp. [İsfendiyaroğlu and Özeker 2001]. However, the effects of species and pretreatments such as GA<sub>3</sub> and stratification and their interaction on the germination percentages were not significant statistically. Similarly, Piotto [1995] reported that there were not significant differences between the scarified and scarified + prechilled seeds of *P. lentiscus*. There are no reports on *in vitro* germination studies of lentisk. Thus, the present study focused on obtaining a rapid reliable and simple method to promote germination of lentisk seeds *in vitro*.

## MATERIALS AND METHODS

**Plant material, culture conditions and establishment.** Mature fruits from open pollinated *P. lentiscus* trees were collected from Çiftlikköy in the vicinity of the Çeşme County in the İzmir province of western Turkey. The soft outer layer of the fruits was removed by hand and they were dried on the laboratory conditions. Seeds were stored in plastic bags at 4°C as the source of plant material for all experiments. Mature seeds, from which the outer pericarp had been removed, were surface sterilized by immersion in a 5% (v/v) sodium hypochlorite solution (Commercial Axion) for 5 min. Then, the seeds washed three times with sterile distilled water before being placed in contact with the full strength MS medium containing 1 mg L<sup>-1</sup> BA and 3% sucrose (w/v) and solidified with agar (0.65% w/v) for ger-

mination unless otherwise stated. Four weeks after culture, the mature seeds of lentisk germinated and the developing seedlings produced actively growing apical shoots. All chemical used were of analytical grade (Sigma Chemical Co. USA). The pH of all media was adjusted to 5.8 before autoclaving (121°C for 15 min). Plant growth regulators (PGRs) were added to the medium prior to adjustment of pH and sterilization. Unless otherwise described, all cultures were maintained in a 16-h photoperiod at  $25 \pm 2^\circ\text{C}$  with irradiance of  $40 \mu\text{mol m}^{-2}\text{s}^{-1}$  provided by cool white fluorescent lamps. All experiments were repeated three times.

**Seed disinfection.** The dried seeds were pre-sterilized in ethyl alcohol (70% v/v) for 40 sec, and washed with sterile distilled water three times and the following surface sterilization experiments were conducted in an attempt to achieve successful culture initiation: (1) effects of different concentration of sodium hypochlorite (NaOCl) (the 5%, 10%, 15% and 20% NaOCl (v/v)  $\times$  20 minute treatments and (2) effects of optimum immersion time in NaOCl (the 5, 15, 20, 30 min  $\times$  5% NaOCl treatments) on the decontamination of lentisk seeds. After washing with sterile distilled water three times, 5 to 6 seeds were placed aseptically in Magenta GA-7 containing 50–60 ml full strength Murashige and Skoog (MS) [1962] medium with Gamborg vitamins,  $30 \text{ g L}^{-1}$  sucrose and  $6.3 \text{ g L}^{-1}$  agar plus unless otherwise stated  $1 \text{ mg L}^{-1}$  BA for viability and contamination studies.

**Effect of media type and strength on seed germination.** To study the effect of different media on seed germination and development of *P. lentiscus* seeds in culture, dehusked fruits (seeds) were surface sterilized and transferred to different full strength media (MS, QL and WPM) supplemented with  $1 \text{ mg L}^{-1}$  BA and 3% sucrose. In a second experiment, in order to examine the effect of medium strength on shoot growth and multiplication, the concentration of nutrients in MS medium was modified from half to full and double strength.

**Effect of different growth regulators on seed germination.** Different plant growth regulators, i.e. BA, GA<sub>3</sub>, NAA and IBA (each one at  $1 \text{ mg L}^{-1}$ ) were used individually in the basal MS medium. Later, on

the basis of their responses in culture the best PGR was used in cultures.

**Effect of different sucrose concentrations on seed germination.** Initially, different sucrose concentrations; i.e. 0, 10, 30, 40, 60 and  $80 \text{ g L}^{-1}$  were used individually in the basal MS medium in order to germinate seeds and for their further growth and development.

**Effect of different period on seed germination and development.** On the basis of the results of the above experiments in culture, the different periods of culture (4, 8 and 12 weeks) were tested in order to find most suitable time for development and growth of plantlets.

**Acclimatization.** The *in vitro* germinated and developed plantlets were washed in running tap water in order to remove agar before being potted in a sterile 1 : 1 : 1 mixture of peat, soil and perlite in plastic cups. These were covered with transparent polythene bags to maintain high humidity during acclimatization for 4 weeks under 16-h light. The plants were irrigated every 2–3 days with water. A four week period of acclimatization by progressive reduction of humidity using sterile compost was used for higher plantlet survival rates. The acclimatized plantlets were transferred to earthen pots in a greenhouse. After 4–5 weeks, the number of plantlets survived in compost was scored.

**Statistical analysis.** Morphological changes were recorded by visual observation. All experiments were carried out in a completely randomized block design. Unless otherwise stated, all experiments were repeated two times and two or three separate blots with at least 15 replicates in each replication. Cultures were incubated for 4 weeks unless otherwise stated. The data on germination (%), shoot length (cm) root length (cm) and leaf number obtained through seed germination were analyzed using standard analysis of variance (ANOVA) procedures and Duncan's multiple range tests as a post hoc comparison of statistical significance (P values  $\leq 0.05$ ). Shoot length (cm) and root length (cm) were measured with a digital compass. The values presented with the percentages were made by comparing the germinated and contaminated seeds to total

seeds cultured. All statistical analyses were performed using SPSS for Windows (version 13).

## RESULTS AND DISCUSSION

**Effects of NaOCl solution on decontamination of lentisk seeds.** Analysis of the data showed that there was evidence of significant difference in the frequency of germinated, contaminated and oversterilised seeds between those that had undergone treatments (tab. 1). The increase of NaOCl concentration from 5% to 20% resulted in a decrease of percentage of seeds contaminated. The lowest contamination (14%) was observed if 15% and 20% solutions of NaOCl were applied. As the NaOCl concentration was increased, the percentage of germination has been decreased, but the rate of oversterilised seeds was increased. Overall, the results presented in Table 1 show no clear combination of providing a level combination suitable for further tissue culture techniques. However, the highest frequency of germinated seed (56%) and the lowest percentage of oversterilised (null) were obtained with the 5% NaOCl treatment. Thus, a 5% NaOCl solution could be tested with further immersion duration on the decontamination of the lentisk seeds.

**Effects of optimum immersion time in NaOCl on the decontamination lentisk seeds.** There was a highly significant difference in the frequencies of the germination and contaminated seeds between those that had undergone the immersion duration in NaOCl solution on decontamination of the lentisk seeds (tab. 2,  $P < 0.05$ ). The highest percentage of germinated seeds was observed after 28 days of culture if seeds were treated with 5% NaOCl for 5 min. The results presented in Table 2 lead to the conclusion that 5% NaOCl  $\times$  5 minute-treatment may be the optimum immersion time to sterilize lentisk seeds.

***In vitro* germination of seeds of *P. lentiscus*: morphological development.** *In vitro* seed germination has not been previously reported for *P. lentiscus*. In our study, the highest germination rates in

four weeks of culture occurred on the full strength MS medium under continued light or a 16 h photoperiod at  $25 \pm 2^\circ\text{C}$ . We examined complete data of the effects of medium type and strength, plant growth regulators, carbohydrate type and different concentrations of sucrose on seed germination, and subsequent seedling development. Seed germination and growth were evaluated after a 4 week incubation period. To reduce variability due to small and deformed seeds, which are least likely to grow normally, equal sized seeds were chosen as far as possible. In a normal healthy seed; the stony pericarp is dark grey or black. Seed size in the study species varied from 2.10 to 2.84 mm. Extraction of hull was relatively simple 1 day after fruit had been harvested. Figure 1A shows a group of immature, mature and dehusked fruits of *P. lentiscus*. Seed germination in lentisk may be separated into two phases: root elongation that occurs within 3–5 day after incubation and a shoot elongation phase that starts 5–7 days after initiation and continues over the culture period. By day 10 a leaf sheath had been formed and by day 15 the first leaf had emerged through the leaf sheath. Four weeks after culture in MS medium containing  $1 \text{ mg L}^{-1}$  BA, root development was slower than that of the leaf. However, within 28 days 4–6 leaf has emerged and associated adventitious buds were clearly discernible.

**Effect of different medium types (mineral solutions).** Media studied increased the *in vitro* germination of lentisk seeds. Significant differences were noted in the efficiency of germination of seeds cultured in the different mineral solutions studied, which ranged from 50% on QL to 66.67% on WPM and 76.67% on MS media, respectively (tab. 3). The results summarized in Table 3 also highlight the efficiency of the nutrient solutions on the germination of *P. lentiscus* seeds. MS medium yielded the highest germination response with the longest shoots and roots, while in QL lower results were obtained than WPM and QL media tested. Nutrient-poor QL medium seemed to be more favorable for root system development.

**Table 1.** Effects of NaOCl treatment on decontamination and survival of the lentisk seeds

Treatment	Germination (%)	Contamination (%)	Oversterilisation (%)
Control	10.0 ±4.28 c	90.0 ±4.28 a	0.00 ±0.00 c
5% NaOCl	56.0 ±7.09 a	44.0 ±7.09 b	0.00 ±0.00 c
10% NaOCl	32.0 ±6.66 b	28.0 ±6.41 c	40.0 ±6.99 b
15% NaOCl	16.0 ±5.23 c	14.0 ±4.95 c	70.0 ±6.54 a
20% NaOCl	10.0 ±4.28 c	14.0 ±4.95 c	76.0 ±6.10 a

Tables presented are the means of 50 replicates observed on 28 day of culture. Means in each column followed by different letters are different according to Duncan's Multiple Range Test ( $P \leq 0.05$ )

**Table 2.** Effects the immersion duration in 5% NaOCl solution on decontamination of the lentisk seeds

Treatment	Germination (%)	Contamination (%)
Control	14.0 ±4.95 b	86.0 ±4.95 a
5 min	60.0 ±6.99 a	20.0 ±5.71 b
15 min	50.0 ±7.14 a	16.0 ±5.23 b
20 min	48.0 ±7.13 a	8.0 ±3.87 b
30 min	46.0 ±7.12 a	6.0 ±3.39 b

Tables presented are the means of 50 replicates observed on 28 day of culture. Means in each column followed by different letters are different according to Duncan's Multiple Range Test ( $P \leq 0.05$ )

**Table 3.** Effect of the culture medium type on lentisk seed germination

Medium type	Germination (%)	Shoot length (cm)	Root length (cm)	Leaf number
WPM	66.67 ±8.75 ab	1.20 ±0.06 a	2.70 ±0.36 a	3.80 ±0.30 ab
MS	76.67 ±7.85 a	1.43 ±0.08 a	3.64 ±0.38 a	4.43 ±0.43 a
QL	50.00 ±9.28 b	1.30 ±0.07 a	3.56 ±0.52 a	3.20 ±0.27 b

Data represent the mean ±SE of 30 seeds per treatment. Values followed by the same lowercase letters within columns are not significantly different at  $P \leq 0.05$

**Table 4.** Effect of strength MS medium on the percentage of lentisk germination

MS Strength	Germination (%)	Shoot length (cm)	Root length (cm)	Leaf number
2	26.67 ±8.21 b	1.37 ±0.18 a	3.25 ±0.49 a	3.75 ±0.41 a
1	70.00 ±8.51 a	1.37 ±0.09 a	3.40 ±0.45 a	4.52 ±0.36 a
½	53.33 ±9.26 a	1.18 ±0.07 a	3.37 ±0.49 a	3.43 ±0.39 a
¼	56.67 ±9.20 a	1.23 ±0.07 a	2.88 ±0.41 a	3.82 ±0.49 a

Data represent the mean ±SE of 30 seeds per treatment. Values followed by the same lowercase letters within columns are not significantly different at  $P \leq 0.05$

**Table 5.** Effect of the plant growth regulators on the percentage of lentisk seed germination

PGR type (1 mg L <sup>-1</sup> )	Germination (%)	Shoot length (cm)	Root length (cm)	Leaf number
Control	46.67 ±9.26 ab	1.34 ±0.07 a	2.97 ±0.25 a	4.33 ±0.39 ab
NAA	40.00 ±9.09 b	0.56 ±0.09 c	1.36 ±0.22 b	2.50 ±0.32 c
BA	73.33 ±8.21 a	1.30 ±0.07 a	3.39 ±0.40 a	4.92 ±0.33 a
IBA	53.33 ±9.26 ab	0.63 ±0.11 bc	0.20 ±0.02 c	3.11 ±0.35 bc
GA <sub>3</sub>	45.65 ±9.15 ab	0.87 ±0.15 b	2.37 ±0.48 a	4.25 ±0.59 ab

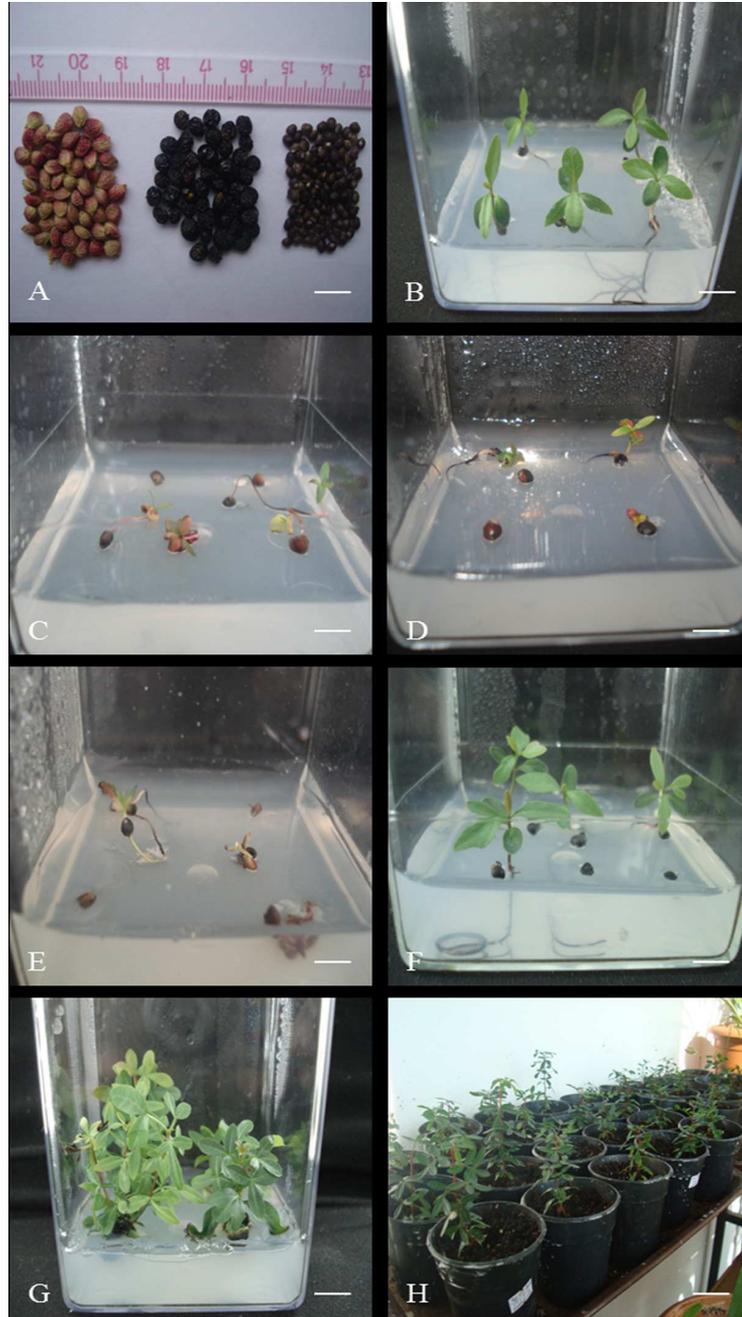
Data represent the mean ±SE of 30 seeds per treatment. Values followed by the same lowercase letters within columns are not significantly different at  $P \leq 0.05$

**Effects of mineral medium strength on seeds germination.** The effects of half, full and double strength nutrients in the culture medium upon seed germination are shown after 28 days of incubation (tab. 4). The seedling development on double strength MS medium seed germination was slow and significantly lower than the other treatments tested. After 30 days of culture, the percentage of seeds germinated was the highest (70%) in the full-strength MS medium. Seeds cultured on full, half and quarter strength media did not differ significantly, whereas the use of double strength nutrient was reduced the percentage of seeds germinated but the germinated seeds showed almost equal development. The strength of the medium used to did not affect the mean shoot length, the mean root length and the mean number of the developed leaves.

Seeds incubated on the full strength medium had more shoots and leaves and longer roots per explant than those on the quarter, half and double strength media. Reducing or increasing the concentration of

nutrient did not have any beneficial effect on shoot length root length or leaf number, whereas decreasing the concentration of nutrients had a negative effect on shoot length and root length, which were not significantly higher on double-strength than on the other treatments tested. The results reported in Table 4 showed that the full strength MS medium induced homogeneous seedling development. However, double strength MS medium seemed to be less favorable for germination.

**Effect of growth regulators.** Data given in Table 5 show that type of plant growth regulators affected significantly percentage of seed germination, and influenced seedling development. From the results, it would appear that the cytokinin BA was superior to other tested treatments in terms of its ability to promote lentisk seed germination and development of seedlings (fig. 1B). It also appears that the tested growth regulators especially the auxin NAA (fig. 1C) and the auxin IBA (fig. 1D) had an inhibiting effect on the healthy germination of seeds compared to GA<sub>3</sub>



(A) Immature (left), mature (middle) and dehusked fruits (right), bar = 8 mm. The effect of the plant growth regulators on the *in vitro* development of seeds after 30 days of culture on the basic MS medium including 0.65% agar supplemented with (B), BA, bar = 8.2 mm; NAA, bar = 6.4 mm (C); IBA, bar = 6.2 mm (D) and  $GA_3$  (E) at concentration of  $1 \text{ mg L}^{-1}$  4 weeks after culture, bar = 6.3 mm; (F) the control treatment, bar = 6.9 mm. (G) Seedling development after 8 weeks in culture, bar = 9.5 mm. (H) The acclimatized plantlets established well upon transfer to a growth room resumed their growth after nine months, bar = 24.5 mm

**Fig. 1.** *In vitro* culture of axenic seeds and development of *Pistacia lentiscus* L. plantlets

(fig. 1E) and the cytokinin BA treatment. The control treatment also yielded a good percentage of germination and in this treatment considerably improved plantlet development (fig. 1F). GA<sub>3</sub> has not been shown to considerably improve germination of lentisk seeds.

**Effect of sucrose concentrations.** The effect of various concentrations of sucrose on seed germination is presented in Table 6. There were significant differences in the frequencies of the germinated seeds among the treatments tested. Thirty days after culture, 36.67%, 76.67%, 66.67%, 70.0%, 36.68% and 20.0% of cultured seeds were germinated on the MS medium supplemented with 0, 1, 3, 4, 6 and 8% sucrose, respectively. According to quantitative evaluation of the germinated seeds, the longest

shoots (1.17 ±0.05 cm) developed on 6% sucrose and the longest roots (0.64 ±0.10 cm) occurred on the 4% sucrose treatment. The lowest mean of root length was obtained for the control group. Through these results, it was observed that no seeds developed with healthy-looking plantlets in the control treatment. Higher concentrations (6 and 8%) clearly decreased seed germination but plantlet growth was similar to each other in all treatments. The present results show that significantly higher rates of lentisk seeds can germinate and grow when dehusked and cultured on MS medium with Gamborg vitamins supplemented with 1 mg L<sup>-1</sup> BA, 30 g L<sup>-1</sup> sucrose and placed in a culture room at 25 ±2°C provided with a 16 h photoperiod through standard fluorescent lamps.

**Table 6.** Effect of the sucrose concentration on germination of lentisk seeds

Sucrose concentrations (g L <sup>-1</sup> )	Germination (%)	Shoot length (cm)	Root length (cm)	Leaf number
Control	36.67 ±8.94 b	0.90 ±0.10 bc	0.36 ±0.03 a	3.27 ±0.50 ab
10	76.67 ±7.85 a	0.87 ±0.07 bc	0.56 ±0.06 a	3.04 ±0.23 b
30	66.67 ±9.24 a	1.05 ±0.05 abc	0.52 ±0.05 a	4.16 ±0.16 a
40	70.00 ±8.51 a	0.82 ±0.06 c	0.64 ±0.10 a	3.28 ±0.20 ab
60	36.68 ±8.92 b	1.17 ±0.05 a	0.60 ±0.15 a	4.18 ±0.12 a
80	20.00 ±7.42 b	1.11 ±0.09 ab	0.45 ±0.07 a	2.66 ±0.42 b

Data represent the mean ±SE of 30 seeds per treatment. Values followed by the same lowercase letters within columns are not significantly different at P ≤ 0.05

**Table 7.** Effect of culture period on axillary bud sprouting, multiplication and acclimatization of axenic stock cultures of *Pistacia lentiscus* L.

Culture period	Shoot number	Shoot length (cm)	Nod number	Viable regenerants four weeks after transferring to growth room (%)	Viable regenerants nine month after transferring to <i>in vivo</i> condition (%)
4 weeks	1.50 ±0.18 b	1.02 ±0.06 b	4.68 ±0.19 c	90.00 ±5.57 a	86.67 ±6.31 a
8 weeks	1.85 ±0.22 b	1.88 ±0.10 a	6.25 ±0.46 b	93.33 ±4.63 a	90.00 ±5.57 a
12 weeks	2.80 ±0.17 a	2.10 ±0.11 a	9.13 ±0.41 a	96.67 ±3.33 a	93.33 ±4.63 a

Data represent the mean ±SE of 30 explants per treatment. Values followed by the same lowercase letters within columns are not significantly different at P ≤ 0.05

**Effect of different period on seed germination and development.** Highest mean shoot number per plant (2.8) was obtained in 12 week period of culture in MS medium containing 1 mg L<sup>-1</sup> BA and this was significantly different than that on the 4 and 8 week culture periods (tab. 7). However, the mean length of the shoots was not significantly different in the 4 and 8 week culture periods. With measured culture periods from 4 week to 12 week, shoot length was also increased and a 12 week period of culture produced the longest shoots (2.1 cm). All the plantlets developed in 4, 8 or 12 weeks culture (fig. 1G) were transferred to a mixture of soil and peat. Shoots grew normally and continued producing additional shoots. The acclimatized plantlets established well upon transfer to a growth room. The method developed for plantlet acclimatization was satisfactory because a high percentage of plant survived (96.67%) in the growth room after a 12 week of *in vitro* culture and 93.33% of these plantlets derived from 12 weeks period of culture resumed their growth after nine months (fig. 1H).

Ex vitro germination of lentisk seeds was poor unless black fruits has been used. Fifty days after sowing, approximately 30% percent of the randomly selected seeds germinated (unpublished results). The color of the fruits is strongly associated with seed viability: black fruits usually contain seeds whereas red ones contain nonviable seeds [Garcia-Fayos and Verdu 1998]. Piotto [1995] reported that neither mechanical scarification of seeds nor prechilling treatments improved the amount of germination of lentisk seeds. Although İsfendiyaroğlu and Özeker [2001] reported a high percentage of seed germination (87.99%) when seeds were pretreated with 1000 ppm gibberellic acid, percentage of germinated seeds was ranged between 0 and 56% in the lentisk population [Mulas et al. 1998] and fifteen ecotypes of 33 (45%) showed absence of seed germination. *P. lentiscus* plays a role of fundamental importance in the conservation and evolution of Mediterranean marquis [Garcia-Fayos and Lolina 1990]. A high

germination rate of lentisk is not only useful for nurseries producing ornamental plants and agroforestry but also for genetic transformation. The results obtained in relation to the different medium types were similar to the findings of Tombolato and Monet [1984] who pointed out that enriched media are beneficial for fresh matter synthesis with respect to peach seeds. Abousalim et al. [1992] showed that low ionic strength culture media promoted growth of *P. vera* plantlets micropropagated from seeds (embryos with their cotyledons). The results reported in Table 4 showed that the full strength MS medium induced homogeneous seedling development.

Interesting results were obtained on the base of the experiment on the effect of growth regulators on seed germination from the presented results; it would appear that the cytokinin BA was superior to other tested treatments in terms of its ability to promote lentisk seed germination (fig. 1B). The auxin IBA (fig. 1D) had an inhibiting effect on the healthy germination of seeds compared to GA<sub>3</sub> (fig. 1E) and the cytokinin BA treatment. Observations have been carried out on *in vitro* germination of *Annona cherimola* Mill. [Jubes et al. 1975, Vargas 1986]. The results of the experiment on the sucrose concentrations show that significantly higher rates of lentisk seeds can germinate and grow when dehusked and cultured on MS medium with Gamborg vitamins supplemented with 1 mg L<sup>-1</sup> BA, 30 g L<sup>-1</sup> sucrose and placed in a culture room at 25°C ±2°C provided with a 16 h photoperiod through standard fluorescent lamps. Similarly Yıldırım et al. [2007] showed that apricot seeds collected in the fall was dormant and obtained a germination percentage of 100% with a vitamin applications and 30 g L<sup>-1</sup> sucrose.

## CONCLUSIONS

In this study, we investigated *in vitro* seed germination and development of *P. lentiscus* plantlets. The results obtained showed that the enriched full strength MS medium supplemented with 1 mg L<sup>-1</sup>

BA and 3% sucrose induced homogeneous and healthy seedling development in a period of 4 to 8 weeks of culture. MS medium without PGR was recommended for profuse and strong rooting while, the combination of peat : soil : perlite (1 : 1 : 1) was chosen as a suitable potting medium for acclimatization. The present protocol of regenerating plant from seeds of lentisk, as outlined in this paper will be a useful tool for reforestation and commercial propagation of new plantations for mastic gum production as well as for genetic improvement of this valuable plant.

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