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Araştırma
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The Effect of Seed Pre-Treatments and Plant Growth Regulators on *In Vitro* Germination of Dormant *Laurus nobilis* L. Seeds*

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Abstract

Objective: In this study, the effect of pericarp and seed coat on germination in dormant Laurel seeds was investigated. In addition, it was aimed to increase the germination rate of Laurel seeds with various pre-treatments and plant growth regulators.

Materials and Methods: Seeds taken from Laurel trees at the Aegean Agricultural Research Institute were used as starting material. Seeds with and without pericarp were cultured in MS nutrient medium. Then, pre-treatments (water and solution of GA_3 soaking) were made to the seeds. After determining the effect of pericarp and pre-treatments on germination, the seeds were cultured in MS nutrient media containing 1 mg/L BAP, GA_3 and NAA.

Results: Germination percentages of 20% and 33.33% and shoot formation percentages 13.33% and 26.67% were found in seeds without pericarp and without pericarp-with a cut seed coat, respectively. As a result of the pre-treatment trials, the highest germination (86.67%), shoot formation (80%), shoot length (2.43 cm), average root lenght (6.81 cm), average leaf lenght (0.73 cm), average number of leaf (2.60 number) were obtained in seeds without pericarp-with a cut seed coat kept in 1000 mg/L GA_3 for 24 hours. In order to determine the effect of plant growth regulators on *in vitro* germination, seeds kept at 1000 mg/L GA_3 for 24 hours were cultured in MS nutrient media containing 1 mg/L BAP, GA_3 and NAA. The highest germination (100%), shoot formation (100%), average shoot lenght (5.13 cm), average root length (7.35 cm), average leaf lenght (0.875 cm), average number of root (2.07 number) and average number of leaf (3.63 number) were obtained in MS nutrient media containing 1 mg/L GA_3.

Conclusion: As a result of the experiments, the dormancy in the Laurel seeds was broken and 100% germination rate was achieved. This protocol provides an effective method for rapid and short-term *in vitro* germination of laurel seeds.

Keywords: Dormancy, germination, Laurel, *Laurus nobilis* L., pericarp, seed coat

Ön Uygulamaların ve Bitki Büyümü Düzenleyicilerinin Dormant *Laurus nobilis* L. Tohumlarının *In vitro* Çimlenmesine Etkisi

Öz

Amaç: Bu çalışmada, dormant defne tohumlarında perikarp ve tohum kabuğunun çimlenmeye etkisi araştırılmıştır. Ayrıca çeşitli ön işlemler ve bitki büyüme düzenleyicileri ile defne tohumlarının çimlenme oranının arttırılması hedeflenmiştir.

Materyal ve Yöntem: Başlangıç materyali olarak Ege Tarımsal Araştırma Enstitüsü'ndeki defne ağaçlarından alınan tohumlar kullanılmıştır. Perikarplı ve perikarpsız tohumlar MS besin ortamında kültüre alındıktan sonra tohumlara ön işlemler (su ve GA_3 solüsyonunda bekletme) uygulanmıştır. Perikarp ve ön uygulamaların çimlenmeye etkisi belirlendikten sonra tohumlar 1 mg/L BAP, GA_3 ve NAA içeren MS besin ortamlarında kültüre alınmıştır.

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Araştırma Bulguları: Perikarpsız ve perikarpsız-kenarına kesik atılmış defne tohumlarında, sırası ile, %20 ve %33.33 oranında çimlenme yüzdesi elde edilirken; %13.33 ve %26.67 oranında sürgün oluşturma yüzdesi belirlenmiştir. Ön uygulama deneyleri sonucunda, en yüksek çimlenme (%86.67), sürgün oluşumu (%80), sürgün uzunluğu (2.43 cm), ortalama kök uzunluğu (6.81 cm), ortalama yaprak uzunluğu (0.73 cm), ortalama yaprak sayısı (2.60 adet) 1000 mg/L GA_3 çözeltisinde 24 saat boyunca bekletilen perikarpsız-kenarına kesik atılmış tohumlarda elde edilmiştir. Bitki büyüme düzenleyicilerinin çimlenme üzerindeki etkisini belirlemek amacıyla, 1000 mg/L GA_3 çözeltisinde 24 saat boyunca bekletilen tohumlar 1 mg/L BAP, GA_3 ve NAA içeren MS besin ortamlarında kültüre alınmıştır. En yüksek çimlenme (%100), sürgün oluşumu (%100), sürgün uzunluğu (5.13 cm), ortalama kök uzunluğu (7.35 cm), ortalama yaprak uzunluğu (0.875 cm), ortalama kök sayısı (2.07 adet) ve ortalama yaprak sayısı (3.63 adet) 1 mg/L GA_3 içeren MS besin ortamında tespit edilmiştir.

Sonuç: Yapılan uygulamalar sonucunda, defne tohumlarındaki dormansi kırılmış ve %100 oranında çimlenme elde edilmiştir. Bu protokol ile defne tohumlarının hızlı ve kısa sürede *in vitro* çimlenmesi için etkili bir yöntem sağlanmıştır.

Anahtar kelimeler: Dormansi, çimlenme, Laurel, *Laurus nobilis* L., perikarp, tohum kabuğu

Introduction

Laurus nobilis L. is an aromatic and medicinal plant belonging to the *Lauraceae* family, which consists of approximately 2500-3500 species (Yilmaz and Ciftci, 2021). *Laurus nobilis* L. is known as laurel, bay laurel, sweat bay, Grecian laurel, true bay, or simply bay (Paparella et al., 2022). Laurel grows up 15-20 meters and it is an evergreen tree or shrub (Boza and Altun, 2013; Ertekin and Corbaci, 2018). Laurel can resist temperatures as low as -15°C (Royandazagh, 2019). The leaves are in a narrow elliptical structure, 5-10 cm long and 2-4 cm wide. Its fruits are egg-shaped, 1 cm in diameter and up to 2 cm long. The fruits are green at first and turn black when ripe (Yilmaz et al., 2014). Generally, flowering begins in April and the laurel flowers are yellow (Sari et al., 2006).

Laurel is generally grown in Mediterranean climates, in Portugal, Spain, Italy, Yugoslavia, Greece, Turkiye and the southern coastal regions of Africa (Ertekin and Corbaci, 2018). Turkiye is the largest producer of *L. nobilis* and exports it to 64 countries. Almost 97% of the world's total production comes from Turkiye. Annual production amount varies between 7000 and 7500 tons (Paparella et al., 2022). Turkiye produces approximately 15000 tons of bay leaves every year. 500-1000 tons of this is used to obtain laurel essential oil. The remaining 14000 tons of bay leaves are exported (Karik and Tuncturk, 2019).

The laurel plant is a highly aromatic plant due to the presence of oil-filled spaces (Royandazagh, 2019). Essential oils obtained from the leaves, bark, fruits and roots of medicinal and aromatic plants constitute an important part of the traditional pharmacopoeia (Kocer et al., 2022). There are different amounts and different types of essential oils in laurel's fruits and leaves (Royandazagh, 2019). There is more oil in ripe fruits than leaves (Yilmaz et al., 2014). The essential oil content of the laurel plant ranges from 0.20% to 2.51%; however, it varies according to factors such as the genotype, the region where it is grown and the harvest time (Golukcu et al., 2018). The laurel plant contains essential oil components such as 1,8-cineol, sabinene, α-terpinyl acetate, α-pinene, β-pinene, methyl eugenol, linalool, terpinen-4-ol, limonene, α-terpineol, camphene, β-myrcene (Kılıc-Pekozlu et al., 2018; Golukcu et al., 2018; Karik et al., 2016; Ercioglu, 2017).

It is known that mankind has benefited from many features of laurel since ancient times (Royandazagh, 2019). Laurel is added to perfume, cream soap and cosmetic materials due to its abundant etheric oils (Paparella, 2022; Ertekin and Corbacı, 2018; Dammak et al., 2019). It has antioxidant, anticonvulsant, analgesic, anti-inflammatory, antiviral, anticholinergic, antibacterial, antiseptic, sedative, antifungal, neuroprotective and wound healing activities due to the essential oils it contains (Kivrak et al., 2017; Souayah et al., 2014; Patrakar et al., 2012).

Laurel can be propagated with seeds or vegetative production methods (Ertekin et al., 2009). In vegetative production of laurel; there are difficulties such as slow rooting and not high rooting rate, rooting ability depending on different times of the year (Parlak, 2007). In addition, due to the dormancy of laurel seeds, production with seeds is difficult. Laurel seeds are covered with a fleshy pericarp and a hard seed coat is found under the pericarp. There are two general types of known seed dormancy: The first is endodormancy occurring due to internal factors such as morphological characteristics, covering structures

or the physiological inhibiting mechanism of the embryo. The second is exogenous dormancy due to the enclosing structures surrounding the embryo (Sari et al., 2006). Laurel seeds have a low germination rate due to the double dormancy resulting from both the seed coat and the embryo (Souayah et al., 2014). There are different methods for breaking laurel seed dormancy and enhancing germination. The most commonly used are soaking seeds in water, remove the pericarp (Konstantinidou et al., 2008), cold stratification, soaking seeds GA_3 (Takos, 2001), using plant growth regulators (Ertekin and Corbaci, 2018).

There are problems in mass seedling production due to the germination barrier of seeds and low success in vegetative production (Ertekin and Corbaci, 2018). In addition, with the increase in the demand for laurel plants, their natural flora are being destroyed (Pirlak, 2012). Because of all this, conservation and amelioration programs are urgently needed to protect the laurel tree (Souayah et al., 2014). Plant tissue culture is a powerful alternative technique for the preservation and reproduction of plants that are rare and difficult to reproduce using conventional methods (Al-Gabbiesh et al., 2014).

Appropriate methods must be applied to break seed dormancy. Impermeability of the seed coat to water or oxygen causes dormancy. In order to break this type of dormancy, the seed coat can be scratched or the seed coat punctured. Physical limitations of the seed coat during embryo development induce seed dormancy. This can be overcome by using mechanical or chemical scarification (Mousavi et al., 2011). In mechanical scarification, the seeds are abraded with sandpaper, abradant or sand. In chemical scarification, the seed coat is removed with chemicals. Acid treatments are often used to break up the thick impermeable seed coats. Since the seeds placed in concentrated sulfuric acid (H_2SO_4) will become charred over time, the temperature of the acid and the soaking time of the seeds are very important. There are different methods for breaking laurel seed dormancy and enhancing germination. The most commonly used are soaking seeds in water, remove the pericarp (Konstantinidou et al., 2008), cold stratification, soaking seeds GA_3 (Takos, 2001), using plant growth regulators (Ertekin and Corbaci, 2018).

The aim of this study is to investigate the effects of different treatments such as removal of the pericarp, soaking in water at different durations, soaking in GA_3 at different concentrations and plant growth regulators such as BAP (Benzylaminopurine), GA_3 (Gibberellic Acid) and NAA (Naphthaleneacetic acid) on the *in vitro* germination of laurel seeds. By evaluating the data obtained as a result of the observations made on the cultivated laurel seeds, a suitable protocol was tried to be developed to break the dormancy.

Materials and methods

Starting Material

Laurus nobilis L. seeds were collected from bay laurel trees (Figure 1) in "Aegean Agricultural Research Institute" in 2022. Seeds were used as starting material for this study.

Figure 1. Laurel trees and seeds at the Aegean Agricultural Research Institute in 2022

Seed sterilization

The seeds were taken into the cabinet and surface sterilization was performed. Sterilization of bay laurel seeds after treatments was carried out by treatment with 70% ethanol for 1 minute and then by treatment with 0.1% $HgCl_2$ for 5 minutes. Sterile water was used 5 times for rinsing.

Nutrient media preparation and sterilization

Nutrient media used in the study were prepared based on semi-solid MS (Murashige and Skoog, 1962). In all experiments, 3% sucrose was used as the carbon source and also 0.3% gelrite as the gelling agent. The pH of the medium was adjusted to 5.8 with 1 M KOH or 1 M HCl. 55 mL glass tubes were used as the culture container and 10 ml of nutrient media was added to each tube. Sterilization of the nutrient medium was carried out by autoclave at 121 ^oC and 1 atm pressure for 15 minutes.

Cutivation of seeds

First of all, the seeds with pericarp, without pericarp and without pericarp-with a cut about 2 mm in diameter on seed coat, shown in Figure 2, were cultured in tubes containing MS nutrient media without plant growth regulators and also any treatments.

These kept in culture room at 24±2 ^oC under a photoperiod of 16 h per day and lighting (3500 lux) provided by white led. At the end of the 6th week, germination percentages were recorded. Applications were performed in triplicate and 10 explants were used for each replication.

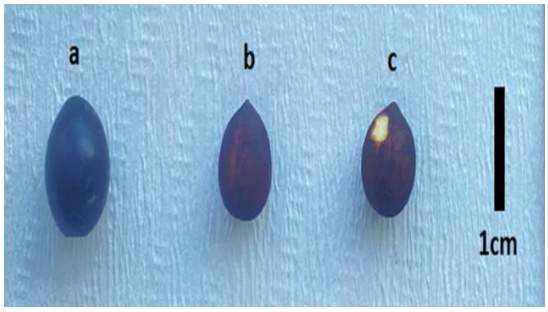

Figure 2. Laurel seeds a. Seed with pericarp, b. Seed without pericarp, c. Seed without pericarp-with a cut on seed coat

In the treatments trials to increase germination, seeds without pericarps and with a cut on seed coat were used. For determining the effect of treatments on *in vitro* germination, seeds were soaked in water (for 0, 24, 48 and 72 hours) and GA_3 (1000, 2000, 3000 mg/L) for 24 hours at room temperature (24^oC). After treatment of seeds, they were cultured in tubes containing MS nutrient media. These kept in culture room at 24±2^oC under a photoperiod of 16 h per day and lighting (3500 lux) provided by white led. At the end of the 6th week, germination percentages were recorded.

After determining the best treatment for germination, the effect of plant growth regulators *on in vitro* germination was investigated. So, seeds were cultured MS based nutrient media containing 1 mg/L BAP, GA_3 or NAA and without plant growth regulators (control) in a sterile cabinet and kept in culture room at 24±2^oC under a photoperiod of 16 h per day and lighting (3500 lux) provided by white led.

Evaluation of data

The results were evaluated 6 weeks after the seed were cultured. The recorded parameters were germination percentages (%), shoot formation percentages (%), shoot length (cm), root length (cm), number of root (number), leaf length (cm) and number of leaf (number).

All the experiments were carried out in 3 replications with 10 explants in each replication according to randomized plots trial design. Minitab 17 Statistical Software (Minitab Inc, PA, USA) program was used to evaluate the obtained data.

Results and Discussion

Effect of pericarp on in vitro germination

According to ANOVA results, pericarp was statistically significant on germination and shoot formation percentages. As a result of the trials to observe the effect of pericarp on *in vitro* germination at the end of the 6th week; 0% germination percentage in seeds with pericarp, 20% germination in seeds without pericarp and 33.33% germination in seeds without pericarps-with a cut on seed coat were obtained. In some of the germinated seeds, shoot formation did not occur (Figure 3). The highest percentage of shoot formation (26.67%) was obtained in seeds without pericarp-with a cut on seed coat (Table 1). In the cultured seeds without pericarp, 13.33% shoot regeneration was obtained. The results shown in the Table 1 indicated that the pericarp completely inhibited the germination of the seed.

Removing the pericarp and cutting the seed coat increased germination compared to germination of seeds with pericarp, but these alone are not sufficient for the germination of the seed. Our research has shown that the pericarp is responsible for dormancy but it is not the only cause of dormancy.

Figure 3. Cultured laurel seeds a. Germinated seed, b. Seed that has germinated but not shoots, c. Seed with shoot

Table 1. Germination and shooting percentages with standard error of the mean (SE) of seeds

	Seed with pericarp	Seed without pericarp	Seed without pericarp-with a cut on seed coat
Germination percentages (%)	0**b**±0	20**a**±5.77	33.33**a**±3.33
Shoot formation percentages (%)	0**b**±0	13.33a**b**±6.67	26.67**a**±3.33

This result suggests that there is embryo dormancy too. Sarı et al. obtained 0% germination of laurel seeds with pericarp, 33% germination of laurel seeds without pericarp and 18% germination of punctured seeds (Sari et al., 2006). Royandazagh found that germination percentage of seeds with pericarp was 0% (Royandazagh, 2019). Takos reported that seed had germination 3% with a pericarp removed knife and germination 35% with a pericarp removed after 10day water soak (Takos, 2001). These results are similar to our germination percentages.

Effect of treatments on in vitro germination

Considering the data in Table 1, treatments were applied to seeds without pericarp-with a cut seed coat. According to their results, seeds germinated between 86.67-33.33% depending on the treatments. Some of the germinated seeds are shown in the Figure 4. According to ANOVA results, treatments was statistically significant on germination percentages, shoot formation percentages, average root length and number of root. The highest germination percentage (86.67%), shoot formation percentage (80%), shoot length (2.43 cm), average root length (6.81 cm), average leaf length (0.73 cm), average number of leaf (2.60 number) were obtained in soaking in 1000 mg/L GA_3 for 24 hour. The lowest germination percentage (%33.33) and shooting percentage (%26.67) were obtained in soaking in water for 0 h (Table 2).

The highest germination percentage (%63.33) were obtained in seeds kept in water for 72 hours. Germination percentage increased with increasing soaking time. However, as the gibberellic acid concentration increased, the germination percentage decreased. The highest germination percentage (%86.67) and shooting percentage (%80) were obtained in seeds kept in 1000 mg/L GA_3 for 24 hours. In Vadochkoriga and Loladze's study, seeds were soaked in water for 48 hours, which caused softening of the pericarp and breaking dormancy (Takos, 2001). In our study, soaking in water increased the germination percentage. These results prove that soaking has a positive effect on breaking seed dormancy. In the study of Sarı et al., a higher germination percentage (34%) was obtained in seeds kept in 1000 mg/L GA_3 for 24 hours compared to other GA_3 concentrations (Sari et al., 2006).

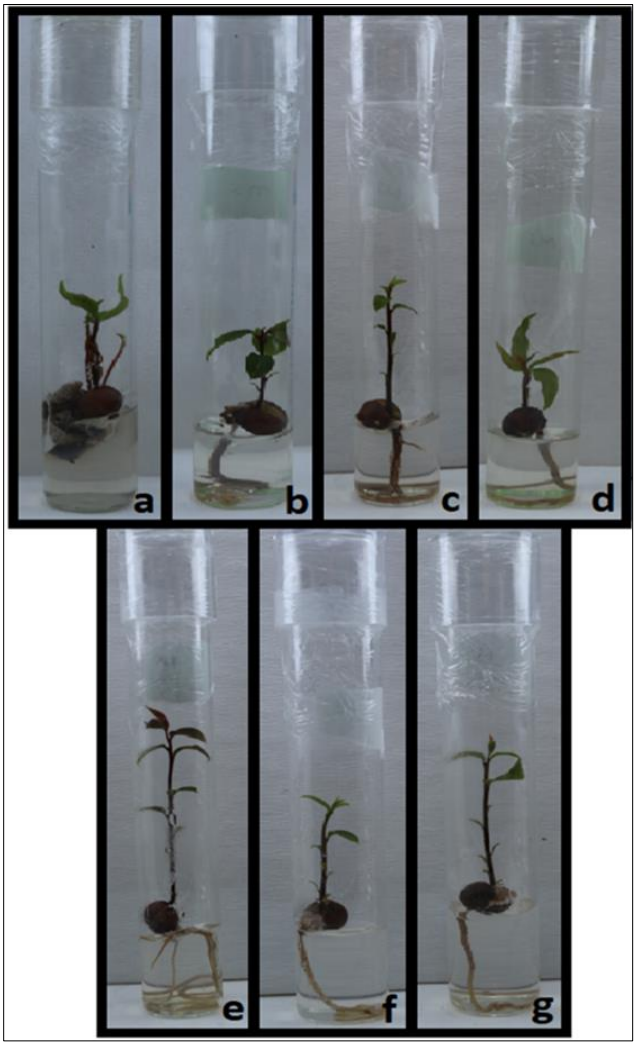

Figure 4. Figure 4. Germinated seeds a. Soaking in water for 0 h; b. Soaking in water for 24 h; c. Soaking in water for 48 h; d. Soaking in water for 72 h; e. Soaking in 1000 mg/L GA_3 for 24 h; f. Soaking in 2000 mg/L GA_3 for 24 h; g. Soaking in 3000 GA_3 for 24 h

Table 2. Effect of different treatments on *in vitro* germination of *Laurus nobilis* L. seeds

Trial	Germination percentage (%)	Shooting percentage (%)	Shoot length (cm)	Average root length (cm)	Number of root (number)	Average leaf length (cm)	Number of leaf (number)
1	33.33**c**±3.33	26.67**c**±3.33	1.25±0.364	1.21**c**±0.431	0.53**b**±0.224	0.34±0.101	1.10±0.357
2	43.33**bc**±14.5	43.33**bc**±14.5	1.10±0.265	3.77**abc**±0.933	0.50**b**±0.142	0.40±0.0956	1.13±0.298
3	60**abc**±5.77	60**abc**±5.77	2.04±0.396	3.14**bc**±0.659	1.03**ab**±0.212	0.38±0.0949	1.43±0.370
4	63.33**abc**±3.33	56.67**abc**±8.82	1.63±0.338	4.36**ab**±0.765	0.70**ab**±0.109	0.37±0.0845	1.93±0.442
5	86.67**a**±3.33	80**a**±0	2.43±0.330	6.81**a**±0.723	1.10**ab**±0.168	0.73±0.0995	2.60±0.425
6	73.33**ab**±3.33	70**ab**±5.77	2.20±0.404	4.79**ab**±0.778	1.43**a**±0.266	0.62±0.108	2.27±0.450
7	70**ab**±0	63.33**ab**±3.33	2.40±0.372	4.94**ab**±0.803	1.30**ab**±0.296	0.57±0.0880	2.43±0.417

*Trial 1: Soaking in water for 0 h (control), Trial 2: Soaking in water for 24 h, Trial 3: Soaking in water for 48 h, Trial 4: Soaking in water for 72 h, Trial 5: Soaking in 1000 mg/L GA_3 for 24 h, Trial 6: Soaking in 2000 mg/L GA_3 for 24 h, Trial 7: Soaking in 3000 mg/L GA_3 for 24 h

Cavusoglu showed that the germination percentage was found as 88.7% in seed with testa kept in 100 ppm GA_3 at 6th week and the germination percentage (81.7%) was achieved in seed without testa kept in 500 ppm and 1000 ppm GA_3 at 12th week (Cavusoglu, 2021). Similarly with these result, higher germination (86.67%) was obtained in seeds kept in 1000 mg/L GA_3 for 24 hours in our study. When the effect of GA_3 on germination is investigated; in the study of Ertekin and Corbaci, increasing the amount of GA_3 decreased the germination percentage (Ertekin and Corbaci, 2018).

In addition, Sarı reported that high GA_3 acid concentrations (3000 mg/L) may be toxic for seeds (Sari et al., 2006). Similar to these data, in this study, as GA_3 concentrations increased, germination percentage and shoot regeneration percentage decreased.

Effect of plant growth regulators on in vitro germination

Taking into consideration the data in Table 3, the seeds without pericarp-with a cut seed coat kept in 1000 mg/L GA_3 were cultured in MS nutrient media containing 1 mg/L BAP, GA_3 or NAA (Figure 5).

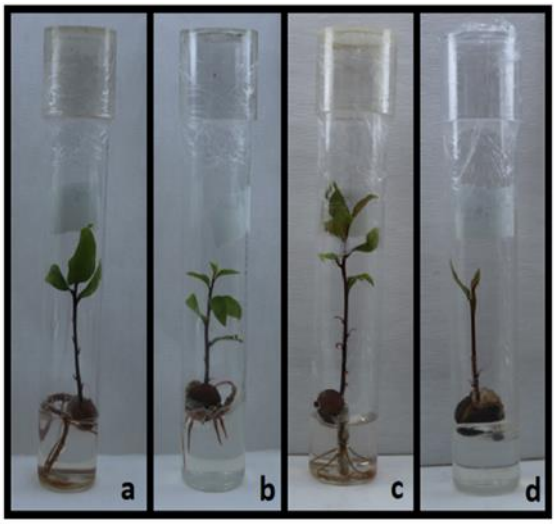

Figure 5. Germinated seeds on MS nutrient media containing different plant growth regulators a. without plant growth regulator; b.1 mg/L BAP; c. 1 mg/L GA_3; d. 1 mg/L NAA

As a result of plant growth regulator trails, the highest germination percentage (100%), shoot formation percentage (100%), shoot length (5.13 cm), average root length (7.35 cm), average number of root (2.07 number), average leaf length (0.875 cm) and average number of leaf (3.63 number) were achieved in MS nutrient media containing 1 mg/L GA_3. In the nutrient medium containing 1 mg/L NAA, 90% germination percentage and 86.67% shoot formation percentage were obtained (Table 3). The lowest germination percentage (%86.67) and shooting percentage (%83.33) was obtained MS without plant growth regulator.

Table 3. Influence of plant growth regulators on *in vitro* germination of *Laurus nobilis* L. seeds

Plant growth regulator	Germination percentage (%)	Shooting percentage (%)	Shoot length (cm)	Average root length (cm)	Number of root (number)	Average leaf length (cm)	Number of leaf (number)
Control	86.67**b**±3.33	83.33**b**±3.33	2.43**b**±0.330	6.81**a**±0.723	1.10**b**±0.168	0.73**a**±0.0995	2.6**a**±0.425
1 mg/L BAP	86.67**b**±3.33	86.67**b**±3.33	2.47**b**±0.281	2.10**b**±0.347	0.97**b**±0.131	0.84**a**±0.0938	3.1**a**±0.443
1 mg/L GA_3	100**a**±0	100**a**±0	5.13**a**±0.457	7.35**a**±0.491	2.07**a**±0.303	0.87**a**±0.104	3.63**a**±0.438
1 mg/L NAA	90**ab**±0	86.67**b**±3.33	1.57**b**±0.226	1.05**b**±0.128	0.90**b**±0.0735	0.29**b**±0.0710	1**b**±0.271

The highest shoot length (5.13 cm) and average root length (7.35 cm) was obtained MS containing 1 mg/L GA_3. The lowest shoot length (1.57 cm) and average root length (1.05 cm) was obtained MS containing 1 mg/L NAA. Cavusoglu and Bozkurt reported shoot length as 1.87 cm and root length as 4.22 cm (Cavusoglu and Bozkurt, 2020). Higher values on shoot length and average root length were obtained in our study.

Compared with BAP and NAA, GA_3 had a positive effect on germination percentage, shoot formation percentage, shoot length, average root length, number of root, average leaf length, number of leaf. NAA increased germination while BAP had no effect on germination. BAP and NAA increased the shooting percentage compared to the control. As a result of Takos's study, best germination result (100%) was

achieved in seed pericarp removed after 10 day water soak and cold stratification in sand (Takos, 2001). In another study, 71% germination was obtained in seeds without pericarp (Parmak, 2019). Chorfi et al. showed that 90% germination percentage, 7.7 cm shoot length and 11.40 cm average root length was obtained in MS nutrient medium (Chourfi et al., 2014). These results are similar to the results obtained in our study.

Conclusion

Due to the essential oils it contains, laurel is used in the treatment of many diseases, as well as; it is also used in the field of cosmetics as a perfume and soap and in the field of food as a spice in meals.The propagation of laurel by traditional methods (with cuttings or seeds) is difficult due to poor fruit formation, very low germination rate, low seed yield and pollination difficulties. Therefore, alternative production techniques are needed for the reproduction of laurel. Since laurel has double dormancy in the laurel, there is a germination barrier. In order to eliminate the germination barrier and enhance the germination, various applications must be made. The pericarp of *Laurus nobilis* L. seeds causes dormancy. But, removal of the pericarp did not completely eliminate the dormancy. Because, there is also embryo dormancy in *Laurus nobilis* L. seeds. Embryo dormancy in seeds without pericarp can be broken by soaking in water, soaking in GA_3, and plant growth regulators. Increasing time of soaking in water has a positive effect on the breaking of embryo dormancy, but increasing the concentration of GA_3 decreased *in vitro* germination. In addition, better germination percentages was obtained in MS nutrient media containing GA_3 compared to containing BAP and NAA. Because gibberellins stimulate the enzymes that play a role in seed germination and eliminate dormancy.

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Conflict of Interest

The authors declare no conflicts of interest.

Authorship contribution statement

HHG: Contributed to the collection of seeds, conducting laboratory studies, making observations, statistically evaluating the results, and writing the manuscript.

AG: Contributed to the execution of the study and writing of the manuscript.

ÜK: Contributed to the collection of seeds and writing of the manuscript.

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