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Dormancy, germination and seedling growth in bay laurel (*Laurus nobilis*)

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Abstract

The present study was conducted to assess the effects of polystimulins, gibberellic acid and duration of cold stratification on germination of bay laurel seeds and seedling growth. Seeds were treated with cold stratification at 4°C for 30, 70 and 90 days; soaking in 500, 750, 1000 mg L^{-1} polystimulins (PS-A6 + PS-K) for 24 hours and then cold stratified for 70 days, or soaking in 500, 750, 1000 mg L^{-1} gibberellic acid (GA_3) for 24 hours and then cold stratified for 70 days. The best results (94%) were recorded when seeds were pre-treated with 1000 mg L^{-1} PS-A6 + PS-K and then cold stratified for 70 days. This study also examined the effects of pre treatment on seedling growth. The greatest seedling height (223 mm) and root length (532 mm) were seen in seedlings produced from PS-treated seeds. Pre-sowing treatment changed the root/shoot ratio of seedlings positively and increased seedling survival. Polystimulin hormone application with cold stratification to seeds is recommended in nursery production of bay laurel seedlings.

Keywords: ornamental plant, germination, plant growth regulator, stratification, seedling growth

Introduction

Bay laurel (*Laurus nobilis* L.) is a plant known since ancient times. It is mentioned in the Odyssey by Homer, and its medicinal qualities were reported by Dioscurides (Skroubis, 1990). It is an evergreen tree or shrub and grows up to 11-m tall. In general, it grows well in Mediterranean climates, for example in Portugal, Spain, Italy, Yugoslavia, Greece, Turkey and the southern coastal region of Africa. Bay laurel is a characteristic tree of the Mediterranean scrub vegetation (Huş, 1966; Baytop, 1991). In Turkey, it grows up to 600-800 m a.s.l. and from Hatay (southeast) to the Black Sea coast (northeast) (Acar, 1987; Anşin and Özkan, 1993).

A characteristic of the *Lauraceae* is that the leaves contain essential oil with a special scent. In addition, the leaves contain cineole, eigenol, α -β pinene and terpineol (Baytop, 1991; Ölmez, 2004). Therefore, the plant is used in the production of soap due to the presence of etheric oils and as an antiseptic in pharmacy due to the high lauric acid content. It is also used in the food industry as a spice and in landscape design (Metcalfe and Chalk, 1957; Tanrıverdi, 1989). In addition, plants are widely used in gardens as solitary plants, in groups or as a green fence because of the green foliage. It is very resistant to air pollution in cities. It can also be used in arid landscapes since it is drought-tolerant (Pamay, 1971; Anşin and Özkan, 1993; Ürgenç, 1998).

Most (90%) of the current production of bay laurel in Turkey is under threat due to over-exploitation. In recent years, some bay laurel plantations have been established in agricultural lands as an alternative product. However, it has been reported that seedlings cannot be produced in sufficient quantity and at a low cost (Bilgin *et al*., 2007; Parlak, 2011; Yılmaz *et al*., 2014; Abacı Bayan, 2016). Generally, stem cuttings and seeds are used for the mass propagation of this species. Other propagation methods are micro-propagation or in vitro culture (Raviv *et al*., 1983; Souayah *et al*., 2002). However, success with vegetative propagation is low (Parlak, 2007), and the presence of germination barriers in seed production (Tilki, 2004) constitutes a problem in mass seedling production for development of plantations. Laurel seeds have dormancy (Sheryshov, 1975; Mkervali, 1977; Vadachkoriya and Loladze, 1986; Takos, 2001). There are different methods for breaking dormancy and enhancing germination (Bewley and Black, 1994; Hartmann *et al*., 1997). The most commonly used are cold stratification and pre-sowing treatments with plant growth regulators (Bonner *et al*., 1994; Nowag, 1998; Ertekin, 2017).

Takos (2001) reported that the pericarp, seed coat and perhaps inhibitor(s) associated with the coat seem to be responsible for laurel seed dormancy. Cold stratification was found to have a positive effect on breaking dormancy in bay laurel, compared with GA_3 and pericarp removal (Takos, 2001; Tilki, 2004; Sarı *et al*., 2006; Çavuşoğlu *et al*., 2014). Tilki (2004) found that, when used alone, gibberellic acid had limited effect on germination of the seeds without pericarp, whereas it significantly increased overall germination when used together with cold-moist stratification. In that research, approximately half of the seeds, which were subject to a 50-day stratification period, germinated (Takos, 2001). Recently, it has been reported that highly bioactive polystimulin-A6 and cytokinin-like polystimulin-K have very positive effects on breaking dormancy, enhancing germination and promoting growth and resistance to environmental stresses (Tsatsakis *et al*., 1993; Ganivea *et al*., 1998; Kırdar and Allahverdiev, 2003; Kırdar and Ertekin, 2008; Ertekin, 2017). Therefore, this study aimed to investigate the effects of different treatments, such as hormones treatment or stratification time, on breaking dormancy of bay laurel seeds.

Material and methods

Bay laurel seeds were collected in October 2014 from naturally-growing trees in a 10-hectare forest area of the Regional Directorate of Forestry in Alaplı-Zonguldak (41°19'N, 31°24'E, altitude 19 m a.s.l.). The trees, from which the seeds were collected were selected randomly with at least 100 m distance between sampled trees. At least 500 seeds were collected from the lower, middle and upper parts of each tree and then all the seeds were mixed. Before the seeds were stratified, they were kept moist in a 0 ± 1°C refrigerator for at least four months without being desiccated (Konstantinidou *et al.*, 2008).

For determining, the effect of pre-sowing treatments on the breaking of seed dormancy, cold stratification (CS) at 4°C was used alone or in combination with hormones. The planned stratification durations were 30, 70 and 110 days. However, during stratification, germination was observed in both the hormone-treated seeds and the control seeds around the 70th and 90th days, respectively. Therefore, the stratification periods used in the research were adjusted to 30, 70 and 90 days. All seeds except control group were stratified in a beaker containing sand moistened with distilled water and stored in a refrigerator at 4°C. For the hormone treatments, seeds were soaked in GA_3 or polystimulins (500, 750 or 1000 mg L^{-1}) for 24 hours at room temperature (22–24°C) and then cold stratified for 70 days at 4°C. Polystimulins (PS) are the synthetic high molecular-weight plant growth regulators, polystimulin-A6 (PS-A6), which is similar to auxin, and polystimulin-K (PS-K), which is similar to cytokinin have high biological activity, similar to 2, 4-dichlorphenoxyacetic acid and 6-benzylaminopurine, respectively, and were defined as plant growth regulators by Tsatsakis *et al*. (1993). PS were used as PS-A6 and PS-K (50% PS-A6 + 50% PS-K).

The following pre-sowing treatments were used:

a) Control
b) CS 30 days
c) CS 70 days
d) CS 90 days
e) GA_3 (500 mg L^{-1}) 24 hours + CS 70 days
f) GA_3 (750 mg L^{-1}) 24 hours + CS 70 days
g) GA_3 (1000 mg L^{-1}) 24 hours + CS 70 days
h) PS (500 mg L^{-1}) 24 hours + CS 70 days
i) PS (750 mg L^{-1}) 24 hours + CS 70 days
j) PS (1000 mg L^{-1}) 24 hours + CS 70 days

Germination tests were carried out in a greenhouse where the average temperature and humidity were 26 ± 0.5°C and 80 ± 5%, respectively, in March 2015 to obtain the same results as nursery practice. Average soil temperature was 21 ± 0.5°C. Photoperiod was 11 hours each day. Seeds were sown in sandy-clay loam at a depth of 2-3 times that of the seed. A random trial design was used with three replicates and 100 seeds in each replicate. Germination percentages were subjected to arcsine-transformation and then ANOVA in SPSS version 16.0 (SPSS Inc., USA) (Zar, 1996). Because of the significant differences in the end of the analysis, Duncan's multiple range test was performed.

To determine the effects of the pre-sowing treatments on seedling growth, seedlings were lifted from the seedbeds in October 2015 and the characteristics of 1-year-old seedlings determined. For this purpose, 30 seedlings were randomly selected from each treatment and seedling height (mm), root collar diameter (mm), root length (mm) and leaf number determined. In February 2016, 150 seedlings (3 × 50) representing each pre-treatment were lifted from the seedbeds and planted in a natural habitat. In October 2016, the percent survival was determined by counting dry seedlings.

In examining the effects of seed pre-treatment on seedling growth, the seedling height (mm), root collar diameter (mm), root length (mm) and leaf number (number) of 30 (3 × 10) seedlings were determined according to a randomised parcel trial design. The Kolmogorov-Smirnov test was used to investigate whether the data were normally distributed. Data that were not normally distributed were square root-transformed before analysis. Duncan's test was used to determine meaningful groups. A Pearson correlation analysis was used to determine the relationships among seedling success for the pre-sowing treated seeds.

Results

Effects of pre-sowing treatment

There were significant differences among pre-sowing treatments in terms of germination percentage (GP) ($P < 0.001$). The highest seed germination (94%) was recorded from treatment-j (PS-A6 + PS-K (1000 mg L^{-1}) + CS 70 days) (figure 1). Germination percentage differed significantly with stratification duration. While 30 days stratification gave 52% germination, 80% germination was obtained after 90 days of stratification. Another pre-sowing treatment, gibberellic acid application, has also been found to be effective on seed germination. In gibberellic acid treatments, which were applied at different concentrations, the highest germination was obtained in treatment-e (GA_3 (500 mg L^{-1}) 24 hours + CS 70 days) with 85%. On the other hand, in gibberellic acid treatments, it has been determined that the germination percentage decreased as the amount of hormones increased.

Effects of seedling growth

There were significant differences among pre-sowing treatments across all seedling characteristics (seedling height, root collar diameter, root length, leaf number and survival percentage) ($P < 0.001$). The highest seedling mean height (221 mm) and the highest seedling mean root length (557.8 mm) diameter were recorded from pre-sowing treatment-j (PS-A6 + PS-K (1000 mg L^{-1}) + CS 70 days) (table 1).

In the correlation analysis, root collar diameter had a negative correlation with seedling height (Pearson correlation: -0.626 and $P < 0.001$). Polystimulin application had positive effects on the morphology of laurel seedlings, including stem-body development and the root/shoot ratio. Seedling survival increased with the root/shoot ratio (Pearson correlation: 0.769 and $P < 0.05$). The highest survival rate (100%) was observed with pre-sowing treatment-j (PS-A6 + PS-K (1000 mg L^{-1}) + CS 70 days), although it did not differ significantly from pre-sowing treatments-j or i (figure 1).

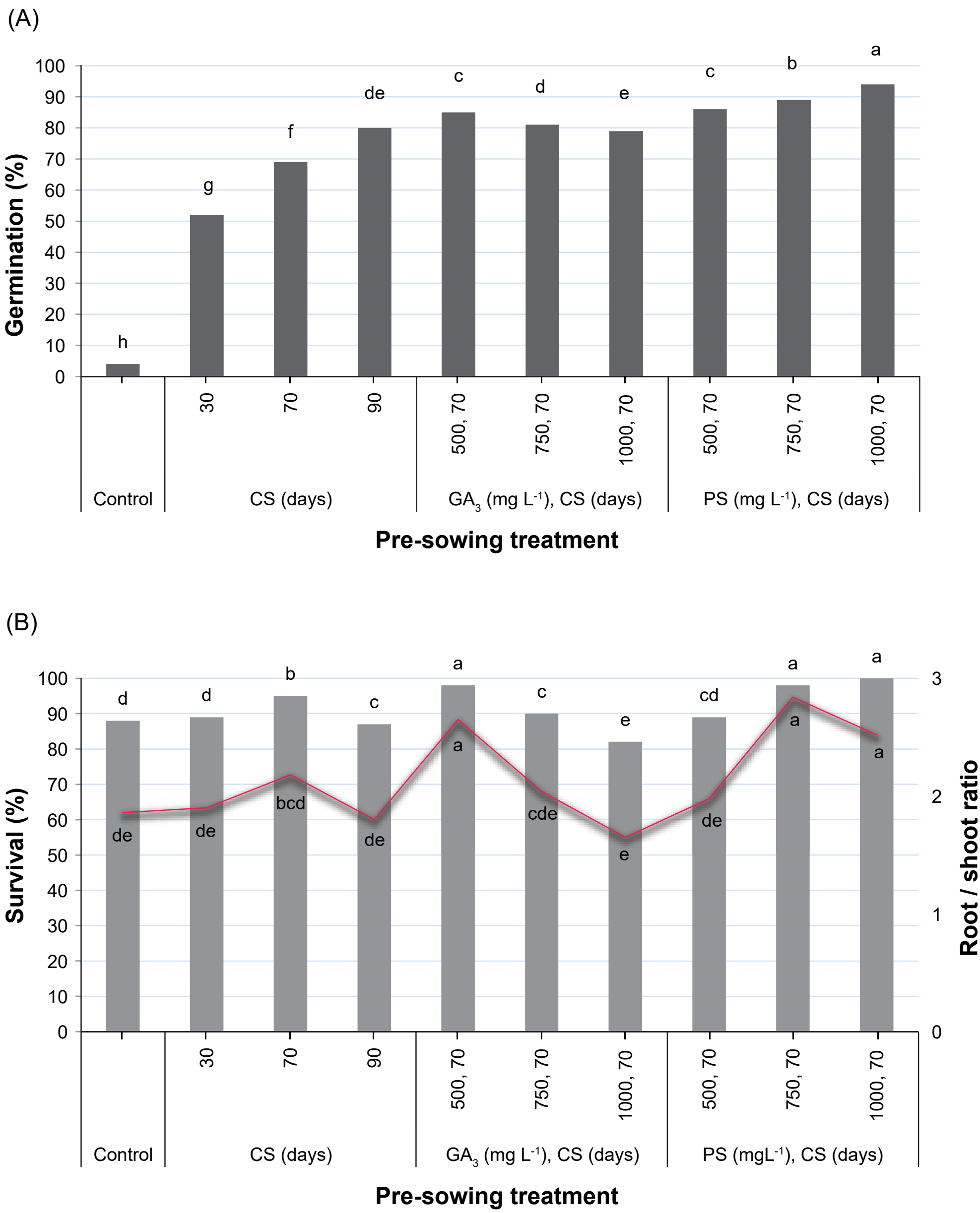

Figure 1. Effects of pre-sowing treatments on seed germination (**A**) and seedling characteristics (**B**) of the bay laurel. Bars with the same letter at the top are not significantly different ($P > 0.05$).

Table 1. Mean values and homogeneous groups according to the Duncan test related to the effects of hormone application and cold stratification (CS) on seedling characteristics of bay laurel. Values with the same letter within a colunm are not significantly different ($P > 0.05$).

Pre-treatments	Seedling height (mm)	Root collar diameter (mm)	Root length (mm)	Number of leaves
Control	137 d	5.75 a	254.8 bc	16.1 c
CS 30 days	143 d	5.76 a	273.3 bc	16.1 c
CS 70 days	146 d	5.75 a	317.0 bc	16.1 c
CS 90 days	145 d	4.61 b	262.0 bc	16.4 c
GA_3 (500 mg L^{-1}) 24 hours + CS 70 days	187 b	4.69 b	494.6 a	19.1 b
GA_3 (750 mg L^{-1}) 24 hours + CS 70 days	165 c	4.69 b	336.7 b	16.1 c
GA_3 (1000 mg L^{-1}) 24 hours + CS 70 days	144 d	4.30 b	238.3 c	14.9 d
PS (500 mg L^{-1}) 24 hours + CS 70 days	159 c	4.27 b	316.3 bc	16.8 c
PS (750 mg L^{-1}) 24 hours + CS 70 days	179 b	4.20 b	508.3 a	19.1 b
PS (1000 mg L^{-1}) 24 hours + CS 70 days	221 a	4.77 b	557.8 a	20.2 a

Discussion

The pre-sowing treatments enhanced the germination percentage of laurel seeds. In this study, the highest GP value (94%) was obtained from pre-sowing treatment-j (PS-A6 + PS-K (1000 mg L^{-1}) + cold stratification 70 days) (figure 1). Looking through the literature, this result (94%) is one of the best values reported for this species. Takos (2001) also reported relatively high germination (85%), if the seed coat is completely removed. In a different study, germination without seed coat was 85%, whereas seeds of the same sample gave 55% after cold stratification for 50 days Sarı *et al*. (2006).

In our study, PS was very effective in breaking dormancy. In the cold stratification treatment with polystimulins, the embryo gained enough growth potential to overcome the mechanical restriction of the seed coat. The other hormone treatment, gibberellic acid, has also been found to be effective on seed germination. 500 mg L^{-1} GA_3 application with cold stratification along 70 days (85% GP) increased germination in comparison with only cold stratification treatment in the same stratification duration (69% GP). On the other hand, as the amount of gibberellic acid increased, the germination decreased. Sarı *et al*. (2006) obtained similar results. Thus, high concentration of GA_3 (3000 mg L^{-1}) may be toxic to the seeds. In another study, Tilki (2004) found that gibberellic acid had little effect on germination of seeds without pericarp when used alone but significantly increased overall germination performance when used with cold-moist stratification.

Cold stratification was successful in overcoming seed dormancy, but it did not produce the highest GP. Moreover, the seed germination was influenced significantly by duration of cold stratification treatment. While 52% germination was obtained from 30 days stratification, 70 days of stratification produced 69% germination. So, in the stratification experiment, the best GP was obtained from 90 days stratification time (80%). Sarı *et al*. (2006) suggested that stratification durations longer than 50 days are needed for higher germination in bay laurel.

In a study of laurel seedling propagation and cultivation, the average height and mean root collar diameter of 1-year-old seedlings of eight different origins were 151 mm and 3.7 mm, respectively (Parlak, 2007). Ertekin *et al*. (2009) reported that pre-sowing treatment affected the growth of laurel seedlings and that the greatest seedling height (212 mm) and root length (508 mm) were obtained from GA_3-treated seeds. In the current study, except for PS application, the seedling heights were generally close to reported results, although the root collar diameters were greater. This might be due to differences in origin (Ertekin *et al*., 2009). PS application improved height and root development in laurel seedlings compared with our other experiments and previously reported data, while the root collar diameter decreased. The greatest root collar diameter was obtained from control seedlings.

In this study, we found that PS application had positive effects on the morphology of laurel seedlings and gave the highest survival rate (100%; figure 1). In the correlation analysis, root collar diameter had a negative interaction with seedling height, and decreased with increasing seedling height. In addition, seedling survival increased with the root/shoot ratio.

Other studies of seedling development obtained similar results for seeds treated with PS. For example, PS positively affected the growth of *Robinia pseudoacacia* L. seedlings, increasing the average seedling height from 730 to 1900 mm (Demircioğlu, 2000). A positive effect on seedling development was also observed in *Magnolia grandiflora* seedlings grown from seeds treated with PS and incubated for 180 days with cold stratification (Kırdar and Ertekin, 2001). In addition to the effects observed following seed treatment, PS had positive effects on seedling development when added to irrigation water (Kırdar and Allahverdiev, 2003). The preliminary treatments applied in the current study also altered the root / shoot ratio of laurel seedlings. In addition, the number of leaves directly affects the yield, which is important in laurel cultivation for leaf production.

Conclusion

In this study, PS application had positive effects on both seed germination and plant growth. It is also considered that the PS application will increase productivity as it increases the number of leaves on bay laurel seedlings, thus, provide economic benefits to the farmers. The use of seeds in massive seedling production is important in terms of conservation and sustainability of genetic diversity. Therefore, it is suggested that similar studies should be carried out for seeds from other bay laurel populations to determine potential differences between populations.

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