

Effect of warm, cold stratification and GA₃ on breaking dormancy and characters germination seeds of *Laurus nobilis*. L in Shahat, Libya

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تأثير المعاملة بالتنضيد (الدافئ – البارد) وحمض الجبريليك على كسر السكون وخصائص إنبات بذور الغار النبيل *Laurus nobilis*. L في مدينة شحات، ليبيا

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Abstract:

This research was conducted to study the effect of cold and warm stratification periods and gibberellic acid concentrations on dormancy breaking and characteristics germination of wild Bay laurel seeds in the archaeological area of Cyrene (Shahat), Al Jabal Al. Akhdar, Libya. The seeds were exposed to stratification periods of 20-40 days (cold - warm), the temperature of cold stratification was 6°C, seeds put in a sterile sand while the warm stratification temperature was 22°C, the seeds were put in layers of moist cotton cloth inside a plastic bag and treated with gibberellic acid concentrations (without, 500 and 1000 mg/L GA₃). Experiment was designed as a completely randomized block design with four replicates. The results revealed that 40 days of cold or warm stratification, with in a 500-mg/L gibberellic acid concentration, achieved the highest germination rate (95%) and improved germination characteristics such as the first day of germination and time spread of germination (17.25–19.00 days, respectively) in addition to germination speed and uniformity (10.42 seed/ day –1.88 day /seed) respectively. In contrast, 20 days of stratification for the both warm and cold without of gibberellic acid resulted of the lowest germination rate, the least speed and uniformity of the germination.

Keywords: Bay laurel, dormancy, warm stratification, cold stratification, GA₃.

الملخص

أُجري هذا البحث لدراسة تأثير فترات التنضيد (الدافئ – البارد) وتركيزات حمض الجبريليك على كسر السكون وخصائص إنبات بذور الغار النبيل البري *Laurus nobilis*. L النامية في المنطقة الأثرية في مدينة شحات بالجبل الأخضر، ليبيا. عُرضت البذور لفترات تنضيد 20 و40 يوماً (دافئ – بارد). كانت درجة حرارة التنضيد البارد 6 °م، وُضعت البذور في رمل معقم، بينما كانت درجة حرارة التنضيد الدافئ على درجة حرارة الغرفة (22 ± 3 °م)، حيث وُضعت البذور في طبقات من قماش قطني رطب داخل علبة بلاستيكية، وُعوملت بتركيزات مختلفة من حمض الجبريليك (بدون إضافة، 500 و1000 ملغم/لتر). صممت التجربة بتصميم القطاعات الكاملة العشوائية بأربع مكررات. أظهرت النتائج أن فترة 40 يوماً من التنضيد (دافئ – بارد) والمعاملة بتركيز 500 ملغم/لتر من حمض الجبريليك، حققت أعلى معدل إنبات (95%) وحسنت خصائص الإنبات، مثل اليوم الأول للإنبات وزمن الإنبات (17.25-19.00 يوماً على التوالي)، بالإضافة إلى سرعة وتجانس الإنبات (10.42 بذرة/يوم - 1.88 بذرة/يوم على التوالي). من ناحية أخرى، كانت فترة 20 يوماً من التنضيد سواءً كان (دافئ- بارد) وبدون المعاملة بحمض الجبريليك أقل معدل للإنبات، وأقل سرعة وتجانس للإنبات البذور.

الكلمات المفتاحية: التنضيد الدافئ، التنضيد البارد، الغار، السكون، حمض الجبريليك.

Introduction

Laurus nobilis L. It has various names Bay laurel, Grecian laurel, Bay Leaf, True Bay, daphne. It is a member of the Lauraceae family [1]The native habitat is the Mediterranean basin. *Laurus* is a dioecious, perennial, evergreen tree or shrub that grows to a height of (4-6) meters. [2,3]. The Bay laurel plant had known since ancient times, as it was mentioned in ancient Greek mythology. Wreaths of laurel leaves decorated the crowns of kings and emperors and were placed on the heads of winners in the Olympic Games as an expression of glory and victory [4]. The Bay tree is a plant of medical, environmental and economic importance, as it is planted as an ornamental tree in parks and public gardens. The leaves are also used to add flavor to many dishes as a type of spice to improve the taste of meat and fish and extend their shelf life [5]. In addition to extracting essential oil from the leaves, flowers, fruits and seeds, which is used in the manufacture of cosmetics, perfumes and soap [6]. Laurel has also been used in medicine since ancient times as a treatment for rheumatism, ear infections, asthma, heart disease, urinary tract infections, diarrhea, as a carminative, diabetes regulator, skin irritation and wounds, antiseptic, antifungal as well as antioxidant [7,8,9]. As of the important the laurel tree, it has become one of the most endangered plant species due to unjust human practices resulting from cutting trees, fires, overgrazing, and land clearing, which has led to a reduction in the areas covered by the laurel [10,11,3]. Currently, the Bay laurel found naturally in a very limited regions in the form of individual trees or simple scattered groups in some locations in the forests of the Jabal Akhdar region. Due to their limited distribution and difficult propagation, a previous study revealed that one of the factors contributing to Bay laurel seed dormancy is the presence of hard seed coats that prevent water absorption, limit gas exchange, and inhibit embryo growth, leading to dormancy [12,13]. Bay laurel seeds are characterized by a low germination rate due to double dormancy caused by both the seed coat and the embryo [14]. Takos and Efthymio (2003) [15] also indicated that germination inhibitors in the outer seed coat prevent water absorption and gas exchange within the seed and reduce protoplasmic permeability. Attributed the failure of Bay laurel seeds germinated due to physiological dormancy in the seed embryo. Many studies conducted on breaking seed dormancy and increasing germination rate, and the most commonly used treatments were cold stratification and treatment with growth regulators [12]. where he used cold stratification, gibberellic acid and bio stimulants to increase the germination rate of Bay laurel seeds, and concluded that all treatments used were superior to the control treatment in germination rate. As a significant of the laurel tree and the necessity of preserving and protecting from deterioration and extinction, this research aimed to study the effect of warm stratification, cold Stratification and gibberellic acid (GA_3) on the break dormancy of the Bay laurel seeds and the possibility of increasing their numbers and planting them in public parks and afforestation of forests with these seedlings.

Material and methods

The fruit collected after full ripening from a wild tree located in the archaeological area of Cyrene (Shahat) at site 32° 49' 30" N, 21° 51' 29" E in began of November 2025. The fleshy pericarp manually removed, the seeds cleaned with running water, dried, and stored in ventilated bags until the study commenced. A total of 240 Bay laurel seeds selected for the experiment and divided between Warm Stratification (W.S.) and Cold Stratification (C.S.) within two periods 20-40 days. Each period Included 60 seeds, distributed among the treatments used (without, 500 and 1000 mg/L GA_3), seeds soaked in gibberellic acid (GA_3) for 24 hours before stratification.

Treatments used:

- 1- W.S 20 days
- 2- W.S 40 days
- 3- W.S 20 days + GA_3 500
- 4- W.S 20 days + GA_3 1000
- 5- W.S 40 days + GA_3 500
- 6- W.S 40 days + GA_3 1000
- 7- C.S 20 days
- 8- C.S 40 days
- 9- C.S 20 days + GA_3 500
- 10- C.S 20 days + GA_3 1000
- 11- C.S 40 days + GA_3 500
- 12- C.S 40 days + GA_3 1000

Cold stratified seeds were placed in clean, well-washed sand, sterilized at 70°C, and distributed into polyethylene bags at a ratio of (2:1) for sand and seeds, respectively. These bags were placed in the refrigerator at a temperature of 6 °C. The first cooling period, for 40 days, began in mid-November 2025. After 20 days, the seeds were introduced for the second cooling period. first warm stratification seeds placed in layers of water-moistened cotton cloth, sterilized with a fungicide, and stored in a plastic container at room temperature (22 ± 3°C). After 20 days of first warm stratification, the second

stratification period was initiated, following the same steps as the first period. At the end of warm and cold stratification periods, the seeds were sown on January 2026. After germination began, the following characteristics were measured:

1- Germination percentage (GP%) = (Number of germinated seeds / Total number of seeds) × 100 according to the equation [16].

2- The first day of germination (FDG)

3- The last day of germination (LDG)

4- Time Spread of Germination (TSG): $SG = (LDG - FDG) + 1$ According to what was stated in [17].

5- Germination speed (GS): The average germination time is calculated by the following formula given by [18]

$$GS = (N_1T_1 + N_2T_2 + \dots + N_nT_n) / N$$

N₁: number of seeds germinating at time T₁

N: Total number of seeds that germinated at the end of the test time

6- Germination uniformity (GU) = Germination Percentage (%) / Number of days to final seedling emergence.

GU = GP / D. According to [19]

GU: Germination uniformity (day/seed)

GP: Total germination percentage (%)

D: Total number of days for seedling emergence.

Statistical Analysis:

The experiment was designed as a completely randomized block design with four replicates. Obtained data were subjected to the statistical analysis of variance (ANOVA) was performed using Costat software. Means were separated the least significant difference (L.S. D) at 0.05 for comparison between means of the different treatments according to [20].

Results

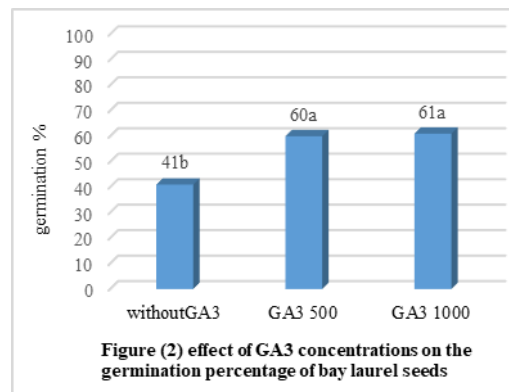
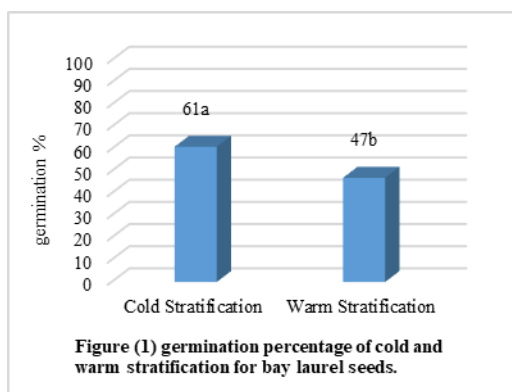
1- Germination percentage (%)

The results in Table (1) indicate significant differences between the studied treatments in the germination rate of Bay laurel seeds. The treatment of 40 days with GA₃ whether warm or cold stratification was significantly differed from the rest treatments. The treatment C.S 40 day + GA₃ 500 gave the highest average germination of Bay laurel seeds (95%) which is nonsignificant form the treatment C.S 40 day + GA₃ 1000 and the W.S 40 day + GA₃ 500 with an average germination (90, 87% respectively). While the treatment W.S 20-day had the lowest values for the average of germination (12%). In general speaking, figure (1) shows a significant difference between the two types of stratification (cold, warm), where cold stratification had highest rate of the germination bay laurel seeds, with an overall average of 61% compared to the warm stratification treatment with an average of 47%. The GA₃ concentrations significantly affected the germination rate (Figure 2), where the concentrations of 500 and 1000mg/L possesses high germination percentage (61 and 60% respectively) which was nonsignificant form each another. While seeds not treated with the GA₃ had the lowest value (41%).

Table (1): Effect of breaking dormancy treatment on germination characteristics of Bay laurel seeds.

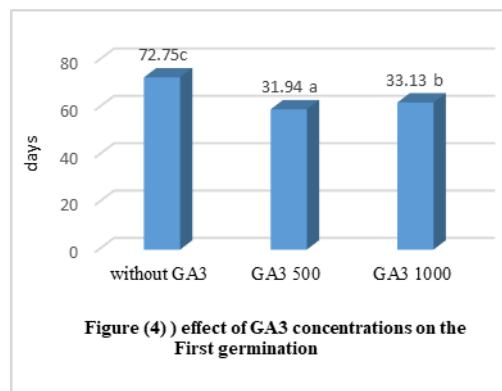
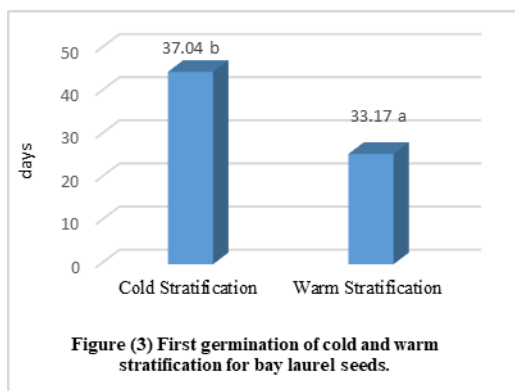
Warm Stratification						
Treatments	GP %	FDG (day)	LDG (day)	TSG (day)	GS (day/seed)	GU (seed /day)
W.S 20 days	12 h	43.00 g	77.50 g	35.5 g	23.22 h	0.42 j
W.S 40 day	57d	28.00 e	55.00 d	28.00 de	14.64 cd	1.18 e
W.S 20 day + GA ₃ 500	15 gh	39.50 f	69.00 ef	30.5 f	18.30 e	0.68 g
W.S 20 day + GA ₃ 1000	30 f	40.50 f	70.00 f	30.5 f	19.55 d	0.58 h
W.S 40 day + GA ₃ 500	87ab	23.00 c	42.00 a	20.00 a	10.42 a	1.88 a
W.S 40 day + GA ₃ 1000	82 bc	25.00 f	50.00 c	26.00 bc	11.02 a	1.64 c
Cold Stratification						
C.S 20 day	25 fg	49.75 i	90 j	41.25 i	22.00 g	0.38 l
C.S 40 day	72c	40.25 f	68.5 e	29.25 ef	15.44 d	1.02 f
C.S 20 day + GA ₃ 500	45 e	48.00 h	84.75 i	37.75 h	21.30 g	0.40 k
C.S 20 day + GA ₃ 1000	42 e	47.00 h	81.25 h	35.25 g	18.41 ef	0.52 i
C.S 40 day + GA ₃ 500	95a	17.25 a	41.25 a	25.00 b	12.26 b	1.76 b
C.S 40 day + GA ₃ 1000	90 ab	20.00 b	47.25 b	28.25 de	13.80 c	1.55 d
LSD 0.05	10	1.51	1.43	1.61	1.18	0.011

Means not sharing the same letter(s) within each column, significantly different at 0.05 level of probability.



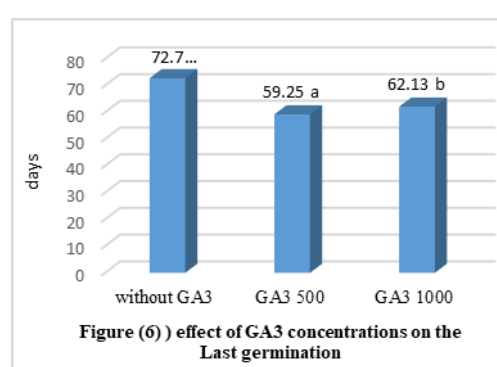
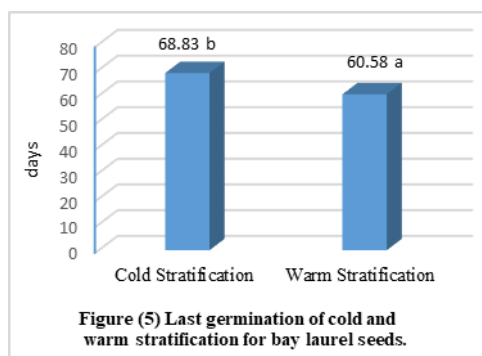
2- The first day of germination (FDG)

The results of Table (1) showed that the studied treatments significantly influence in first germination day of noble bay seeds. The treatment C.S 40 day + GA3 500 was earlier than the rest treatments (17.25), followed by the treatment C.S 40 day + GA3 500, with an average of 20 days from the planting. In contrast, delay seed germination in treatments stratified for 20 days, with or without GA3 where, germination began after planting by 49.75 days in the C.S 20-day treatment. Also, figure (3) indicates a significant advantage of warm stratification in the first day of germination, with an average of 33.17 days compared to cold stratification. Furthermore, there are significant differences between the concentrations of GA3 in the initial germination stage, where the 500mg/L GA3 concentration achieving an average of 31.94 days, compared to the other concentrations. The seeds without GA3 were delayed in their initial germination stage, with an average of 40.25 days figure (4).



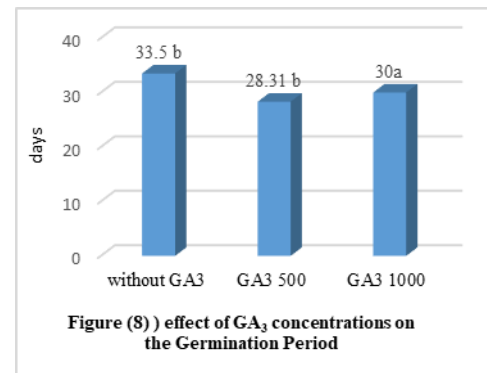
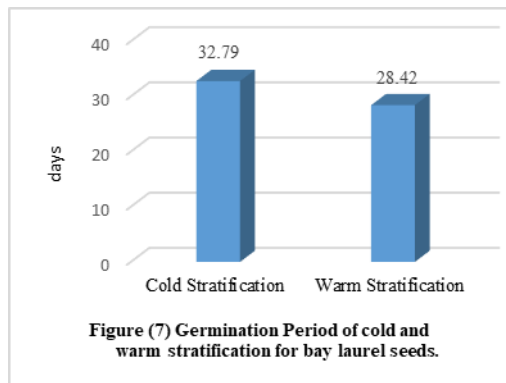
3- The last day of germination (LDG)

The results of Table (1) the treatments stratified for a period of 40 days (cold - warm) and treatments with a concentration of GA3 500 were significantly superior in last germination trait to the rest of the treatments with an average of 41.25, 42.00 days and its did not differ significantly between them, while the seeds took 90 days until reach end germination in the treatment C.S 20 day. Figure (5) also shows that warm stratification was significantly superior in the trait of the last day of germination, with an average of 60.58 days, compared to cold stratification (68.83). Furthermore figure (6) shows that the GA₃ concentration of 500 mg/L had the less last day of germination with an average of 59.25 days, compared to the rest concentrations used.



4- Time Spread of Germination (TSG)

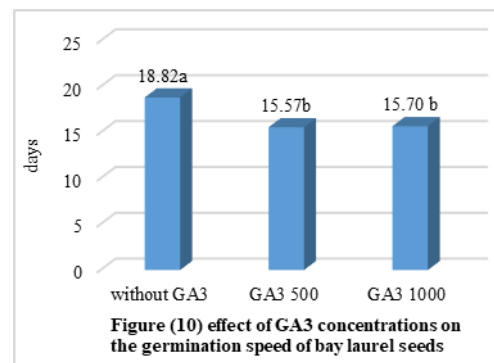
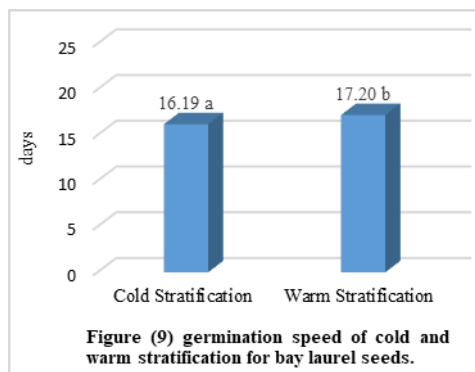
The results in Table (1) show significant differences between the studied treatments in seed germination period. The treatment W. S 40 day + GA₃ 500 significantly different to other treatments, which is taking only 20 days from first to last germination. The treatment C.S 40 day + GA₃ 500 also improved germination Period by an average of 25 days, while seeds treated with C.S 40 day required 41.25 days from first to last germination. In addition, when comparing between cold and warm stratification (finger 7) found that the cold stratification had the lowest TSG within average of 32.79 days, while the figure (8) revealed the lowest average was at concentration 500 mg/L (28.31, days).



5-

Germination Speed (GS)

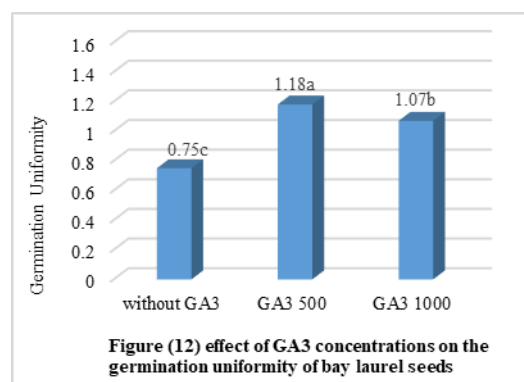
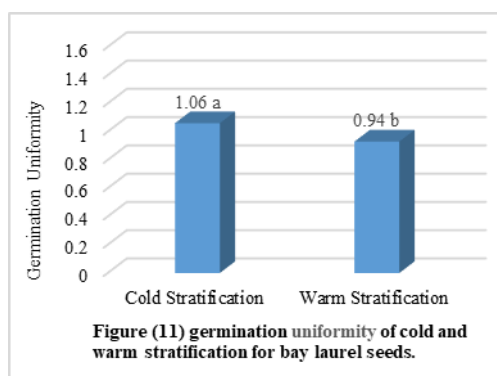
The studied treatments significantly affected seed germination speed (Table 1). The 40-day stratification warm with concentrations of 500 and 1000mg/L resulted in the fastest seed germination rates. The treatment W. S 40 day + GA₃ 500 and C. S 40 day + GA₃ 1000 achieved the fastest average seed germination rate (10.42,11.02 days/seed, respectively). In contrast, the 20- day warm stratification treatment (W.S 20 day) resulted in the slowest seed germination rate, with an average of 23.22 (days/seed) . The figure (9) shows that cold stratification significant differ from warm stratification in germination speed, with an overall average of 16.19 days/seed, compared to warm stratification, with an overall average of 17.20 days/seed. Also, there was a significant difference between the GA₃ concentrations used, with untreated seeds showing the slowest germination rate (18.82), while the 500 and 1000mg/L concentrations had the highest average germination rate 15.57, 15.70, which nonsignificant among them figure (10).



6- Germination Uniformity:(GU)

The studied treatments significantly impact in seed germination uniformity (Table 1). Seeds stratified for 40 days and treated with GA₃ concentrations (cold-warm) significantly differ from the rest treatments. The W.S 40 day + GA₃ 500 treatment achieved the highest germination uniformity with an average of 1.88 seeds/day, followed by the C.S 40 day + GA₃ 500 with average 1.76 seeds/day. In contrast the W.S 20-day treatment had the lowest germination uniformity (0.38 seeds/day). In addition, figure 11 shows that cold stratification was significantly superior in seed germination uniformity to warm stratification with an overall average of 1.06 seeds/day, while warm stratification had the lowest germination uniformity with an overall average of 0.94 seeds/day. moreover, the 500mg/L GA₃ concentration significantly affected germination uniformity (Figure 12)

achieving the highest average germination uniformity (1.18 seeds/day) which significantly differ from other concentrations used.



Discussion

Bay laurel seeds are characterized by double dormancy due to the seed coats and physiological embryo dormancy [13], which causes a decrease in the germination rate, as indicated by previous studies. This led to the use of stratification treatments (cold - warm) and concentrations of GA₃ to improve the characteristics and properties of seed germination. According to the results obtained, the treatment led to the treatment C.S 40 days + GA₃ 500 recorded the highest germination rate of 95%. These results are consistent with Ertekin (2018) [12], who found that bay seeds germinated for 70 days with cold stratification achieved a germination rate (94%), and also with the study by Fadi Kazngi and Talal Amin (2025) [21]. The germination rate reached 98%. These results may be attributed to biochemical changes in the total sugar and starch content of the seeds during stratification, these changes activate the hydrolytic enzymes amylase, protease, and lipase, leading to the conversion of complex compounds such as carbohydrates and proteins into substances easily absorbed by the embryo, it also contributes to the rapid transition of the embryo growths, thus breaking dormancy, speed and uniformity germination [22,23]. Soluble sugars also act as regulatory signals for gene expression during the breaking of dormancy [24]. Also, soluble sugars activate heat-sensitive proteins, affecting the properties of cell membranes, thus altering the thermal sense and reducing the activation energy necessary for growth and germination. Stratification also increases the levels of endogenous gibberellins and cytokinin, thereby reducing the effect of inhibitors such as abscisic acid (ABA) [25,22]. Notably, the cold stratification period decreased complex compounds in the seed coat such as lignin and total phenol contents. Moreover, treatment with GA₃ positively affected in shortening the stratification period and breaking dormancy, where the concentrations of 500 and 1000mg/L had high germination percentage (61 and 60% respectively) compared to without GA₃, moreover enhancement of all other traits. The role of gibberellic acid (GA₃) in improving germination can be explained by influence of enzymatic synthesis of complex-digesting enzymes (sugars, proteins, lipids) and ribonucleases, which are subsequently used to re-encode new types of RNA which are used in the germination process [26,27]. GA₃ plays a role in hydrating the endosperm, reducing seed hardness, and accelerating the breakdown of complex compounds in the seed coat [28].

Conclusion

Due to the medicinal, environmental, and economic importance of the bay tree, the difficulty of propagating it, the need to protect it from extinction and decline, as well as the low seed germination rate resulting from double dormancy caused by both the seed coat and the physiological dormancy of embryo, cold stratification treatments at 6°C and warm stratification treatments at room temperature (22°C) for 20-40 days, along with soaking in gibberellic acid concentrations of 500-1000, were used to break seed dormancy and increase the percentage and speed of germination. The stratification treatment, whether cold or warm, for 40 days, combined with treatment with a 500 mg/L concentration of gibberellic acid, proved to be the most effective treatment for breaking seed dormancy and increasing the germination rate and uniformity. Further studies on bay seeds are suggested, such as the use of natural germination stimulants to determine their effect on accelerating and uniform germination and Vegetative propagation by cuttings.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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