HortScience 60(6):906–909. 2025. https://doi.org/10.21273/HORTSCI18494-25

Germination Enhancement and Phytohormonal Dynamics in Sandalwood Seeds

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Keywords. dormancy, gibberellic acid, *Santalum album*, seed germination, stratification

Abstract. **Sandalwood (*Santalum album* L.) is a species with high economic value due to its fragrant heartwood and essential oil. Its propagation, however, can be challenging due to low and sporadic seed germination. This study aimed to investigate the effects of presowing soaking in distilled water or gibberellic acid (GA_3; 500 ppm) either with or without cold stratification (seeds kept in moist peatmoss at 4 °C for 30 days before sowing). Data were recorded on germination percentage (G%) and germination rate index (GRI), together with phytohormonal analysis of seeds using high-performance liquid chromatography. Results revealed that GA_3 treatment significantly enhanced both G% and GRI compared with other treatments, attaining the highest G% (68.07%) and GRI (21.86) in GA_3-treated seeds. Conversely, cold stratification reduced germination metrics and levels of promotive phytohormones. Phytohormonal analysis indicated elevated levels of GA_3 and indole-3-acetic acid in GA_3-treated seeds, correlating with improved germination. The study emphasizes the potential of GA_3 in overcoming seed dormancy and provides a foundation for sustainable sandalwood cultivation.**

Sandalwood refers to a class of woods from the genus *Santalum*, commonly known as Indian sandalwood. It is a species of flowering tree in the family *Santalaceae* native to southern India and Southeast Asia. This tree is highly valued for its fragrant heartwood, which produces sandalwood oil, a significant material in perfumery, cosmetics, and traditional medicines. The heartwood and its essential oil are renowned for their distinctive and pleasant fragrance that has been highly prized for centuries (Cottrell 2022). Once germinated, the young seedlings require a host plant to support their growth, making the cultivation of sandalwood somewhat complex but rewarding due to its valuable heartwood and oil, which are extensively used in perfumes, beauty products, and traditional medicines, contributing significantly to local and global markets (Nakandalage et al. 2021). Environmentally, sandalwood trees play a crucial role in maintaining biodiversity because they form symbiotic relationships with other plant species, enhancing soil health and stabilizing ecosystems. Moreover, sandalwood plantations can provide sustainable income for rural communities, promoting rural development and reducing pressure on natural sandalwood forests, thereby contributing to conservation efforts. However, the propagation of sandalwood can be challenging, with seed germination being a crucial problem. The seeds have a hard outer coat, which can delay germination (Cottrell 2022; Das 2021).

To address these challenges, various presowing techniques, such as soaking in water, gibberellic acid (GA_3), and various bioregulators, as well as seedcoat scarification, have been suggested to enhance germination rates. Several authors have reported poor germination of sandalwood seeds, with sporadic and irregular germination over a period extending to almost a year with 1 month as the minimum time required for the emergence of the first germination. To understand the nature of dormancy in sandalwood seeds, previous literature suggested that the impenetrable seedcoat or chemical substances (Joshi et al. 2022). However, a study conducted by Jayawardena et al. (2015) revealed that dormancy in sandalwood seeds could be classified as a non–deep morphophysiological type as deduced from the germination enhancement by presowing GA_3 treatment. They excluded the seedcoat as being a physical barrier according to their observation of readily imbibition in scarified and nonscarified seeds. In contrast, more recent studies such as Hemalatha and Chaudhari (2020) confirmed the need for scarification in combination with GA_3 treatment to release dormancy in sandalwood seeds. Furthermore, the deepness of dormancy in sandalwood seeds was found to be highly affected by the source of seeds (Shankar and Devakumar 2018), as well as seed size and the stage of fruit development (Manonmani and Vanangamudi 2002). Controversial results in relevant literature on the germination of sandalwood raise the issue of the need for further studies on germination considering tracing the changes in seed phytochemicals throughout the germination stages to gain a better understanding of the nature of dormancy and the effect of various treatments on breaking dormancy in sandalwood seeds.

Accordingly, the aim of this study was to examine the impact of presowing treatments including the combination of cold stratification and soaking in water or GA_3 on breaking *Santalum album* seed dormancy and promoting germination in addition to the levels of phytohormones.

Materials and Methods

This experiment was conducted at the Department of Agriculture, Faculty of Environmental Sciences, King Abdulaziz University, Saudi Arabia. The mature, ripe, and uniform seeds of sandalwood were collected from the mother plant in Jazan region. During the drying process, the seeds were treated with fungicide to prevent fungal growth. Five treatments were applied to sandalwood seeds: T1—water soaking, T2—GA_3 500 ppm, T3—water soaking + cold stratification, and T4—GA_3 soaking + cold stratification. For soaking in water or GA_3, seeds were soaked for 24 h before sowing or undergoing cold stratification. Cold stratification was applied by keeping seeds in moist peatmoss under cold conditions (4 °C) for 30 d before sowing.

For all treatments, seeds were sown in multicell plastic trays containing a 1:1 mixture of peatmoss and perlite. To maintain a sufficient level of humidity during the germination stage, the trays were kept under shade conditions (70% shade) and covered with a plastic sheet. The experiment was laid out in a completely randomized design with three replications. In each replication, 100 seeds were sown for each treatment. The number of germinated seeds was recorded daily, and the total number of germinated seeds was registered when the experiment was ended. Germination indices were then calculated according to the formulas cited by Hemalatha and Chaudhari (2020) and Shankar and Devakumar (2018). The germination percentage (G%) was calculated using the formula: G% = number of seeds germinated/total number of seeds planted × 100. The germination rate index (GRI) was also calculated according to the formula: GRI = G1/1 + G2/2 + …. + Gx/x (G denotes the germination percentage on the corresponding day). Higher GRI indicates that seeds germinated quickly, uniformly, and consistently.

Quantitative analysis of promotive and retardant phytohormones in the treated seeds was conducted using high-performance liquid chromatography (HPLC) analysis. Identifying and quantifying phytohormones was done in alcoholic extracts of seeds using HPLC

Received for publication 31 Jan 2025. Accepted for publication 7 Mar 2025.
Published online 8 May 2025.
This project was funded by KAU Endowment (WAQF) at King Abdulaziz University, Jeddah, under grant no. WAQF: 183-155-2024. We thank WAQF and the Deanship of Scientific Research for technical and financial support.
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(Agilent 1100; Agilent, Santa Clara, CA, USA) coupled with two LC pumps, a ultraviolet-visible detector, and a C18 column (125 mm × 4.60 mm, 5-µm particle size). The mobile phase used was composed of methanol (solvent A) and acetic acid in water at a 1:25 ratio (solvent 2). The eluting compounds were detected at a range from 250 to 700 nm wavelengths. The gradient program began with 100% B and was held at this concentration for the first 3 min. This was followed by 50% eluent A for the next 5 min, after which the concentration of A was increased to 80% for the next 2 min and then reduced to 50% again for the following 5 min. The obtained chromatograms were analyzed using the Agilent ChemStation, and the final concentrations were expressed as micrograms per gram of dry seeds. The detected compounds were identified and quantified with standard curves of authentic chemicals purchased from Sigma-Aldrich (St. Louis, MO, USA). These authentic compounds included GA_3, 6-benzylaminopurine (BAP), indole acetic acid (IAA), and abscisic acid (ABA).

Statistical analysis. One-way analysis of variance was applied to detect differences between treatments (n = 5). The mean values were compared using the least significant difference test at a P value of 0.05. All data were presented as mean ± standard deviation. The analysis was performed using Statistix software (ver. 8.1; Analytical Software, Tallahassee, FL, USA).

Results

Comparing seed-soaking treatments without stratification, it is clear that GA_3-treated seeds exhibited the highest germination percentage (68.07%), outperforming the water-treated group (43.33%) with significant differences (P = 0.05) (Fig. 1). When stratification was employed, the same trend was also noticed where GA_3-treated seeds with stratification (15.3%) surpassed the water-soaked seeds with stratification (9.7%). However, the combined stratification treatments (water + stratification and GA_3 + stratification) significantly reduced germination percentages (9.70% and 15.30%, respectively) compared with the control and GA_3-only treatments. Accordingly, stratification may not be beneficial for sandalwood seed germination.

The GRI showed a similar trend as the germination percentage (Fig. 1). The highest GRI (21.86) was shown by seeds treated with GA_3 with significant differences (P = 0.05) compared with those subjected to any of the other treatments, suggesting that GA_3 considerably increased the speed and consistency of germination. Compared with the treatments without stratification, both stratification treatments (water + stratification and GA_3 + stratification) produced noticeably reduced GRIs (1.02 and 3.06, respectively). This implies that stratification might prevent sandalwood seed germination. These outcomes provide compelling evidence for the ability of GA_3 treatment to improve sandalwood seed germination. The notably higher GRI that GA_3 yielded indicates that it facilitates synchronous germination and speeds up the germination process, which results in more uniform and quicker seedling establishment.

To help understand the positive effect of GA_3 in enhancing the rate and speed of seed germination, a quantitative analysis of both promotive and retardant phytohormones in the treated sandalwood seeds was done using HPLC. Data illustrated in Figs. 2 and 3 reveal the detection of GA_3 and one auxin; IAA and one cytokinin; and BAP in addition to ABA. Retention times for these compounds were as follows: GA_3 = 2.9 min, BAP = 4.9 min, IAA = 6.0 min, and ABA = 7.9 min. GA_3 concentration, on the other hand, significantly varied between treatments. The seeds treated with GA_3 had the highest concentration of GA_3 (19.05 µg/g), which corresponds almost to double that in water-treated seeds (9.05 µg/g). However, the effect of stratification was contrastive; it led to a significant decline in GA_3 concentration in GA_3-treated seeds (5.17 µg/g) but had a slight or no effect on water-treated seeds (9.29 µg/g). Regarding the auxin IAA, its concentration showed a similar pattern to GA_3 and was highest in GA_3-treated seeds (19.73 µg/g) surpassing that in water-treated seeds (14.22 µg/g). Stratification, however, negatively affected IAA concentration in GA_3-treated seeds, whereas it did not affect the water-treated ones. The cytokinin BAP had a more similar performance to IAA; its concentration in GA_3-treated seeds surpassed that in all other treatments. Also, stratification treatment did not affect BAP concentration when seeds were treated with water, although it led to a significant decrease in its concentration in GA_3-treated seeds. Although ABA, a germination inhibitor, was present in all treatments, the differences were not significant. This indicates that ABA is unlikely to be responsible for reduced germination in stratification-treated seeds.

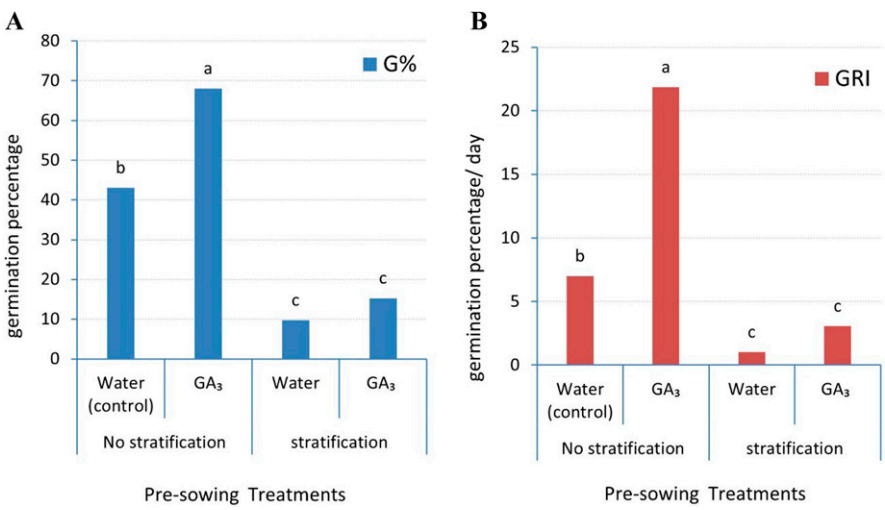

Fig. 1. Effect of presowing treatments of germination of sandalwood seeds: (**A**) cumulative germination percentage (G%) and (**B**) germination rate index (GRI). Different letters above columns denote significant differences among the four treatments at P = 0.05 using the least significant difference test. GA_3 = gibberellic acid.

Discussion

The findings of the current study emphasize the effectiveness of GA_3 presowing treatment in disrupting dormancy and enhancing the germination rate of sandalwood seeds. These results are in alignment with the findings of a recent study conducted by Polaiah et al. (2020) on sandalwood seeds, who reported the superiority of GA_3 treatment compared with the other presowing treatments attaining the highest germination percentage (41%) of sandalwood. However, they reported the lowest germination percentage (6.66%) in the control seeds treated with sulfuric acid, suggesting that the embryos might have been severely damaged by this treatment. Likewise, Jayawardena et al. (2015) recorded a significantly higher germination rate in sandalwood seeds treated with GA_3 at 500 ppm in both nonscarified and scarified seeds, with no significant difference between treatments. The same treatment was also reported as superior by Shankar and Devakumar (2018), with the seed source having a significant influence. Hemalatha and Chaudhari (2020), however, suggested the need for scarification in combination with GA_3 treatment to enhance the release of sandalwood seed dormancy. On the other hand, cold stratification treatment, either alone or combined with GA_3, showed a negative effect on germination and hormonal balance in seeds. This may suggest that stratification is not suitable for overcoming seed dormancy in sandalwood because it reduces the levels of promotive phytohormones.

To explain these results, two general effects have been suggested for GA_3 to promote germination, which include facilitating water absorption and cellular elongation through enzymatic activation and structural modification. The production of enzymes such as -amylase, which hydrolyzes starch and provides energy for embryo growth, is encouraged by gibberellins (GAs) (Nautiyal et al. 2023). In

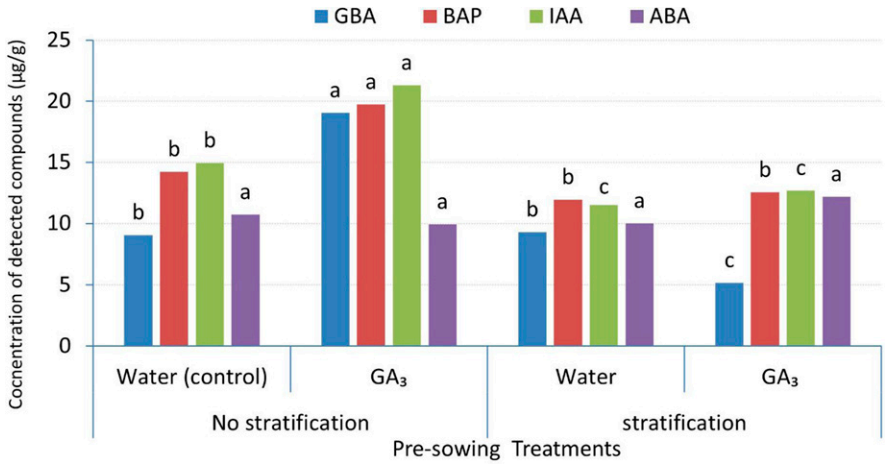

Fig. 2. Quantitative high-performance liquid chromatography analysis of promotive and retardant phytohormones in the sandalwood seeds treated with presowing soaking treatment in water or gibberellic acid (GA_3) at 500 ppm either with or without cold stratification treatment. Different letters above columns denote significant differences among the four treatments for the corresponding detected compound at $P = 0.05$ using least significant difference test. ABA = abscisic acid; BAP = 6-benzylaminopurine; GBA = gibberellic acid; IAA = indole acetic acid.

addition, GAs assist in the synthesis of other structural proteins, which is connected to improved embryonic development. On the cellular level, GAs are important for cellular elongation through triggering pectinase activity and thus increasing cell wall plasticity and water absorption required for seed imbibition, which leads eventually to the initiation of seed germination. The effect of GAs is not restricted to the conditions surrounding the embryos; however, they directly affect the growth of the radicle by accelerating cell division and elongation. GA-dependent expression of cell wall–related enzymes during germination has already been reported for other species such as yellow cedar (Ren and Kermode 2000) and tomato (Chen and Bradford 2000; Nonogaki et al. 2000) and *Arabidopsis* (Ogawa et al. 2003).

The hormonal analysis further elucidated the interplay between phytohormones during germination. The higher concentrations of gibberellins (GA_3) and auxins (IAA) in GA_3-treated seeds correlated with improved germination metrics, whereas the inhibitory hormone ABA showed no significant variation across treatments. This highlights the dominant role of gibberellins and auxins in overcoming dormancy and promoting germination. In agreement with our results, Ogawa et al. (2003) found that exogenous application of GA led to a significant increase in auxin levels during *Arabidopsis* seed germination. They attributed this to the upregulation of genes responsible for auxin carrier proteins, suggesting that GA may enhance auxin biosynthesis as well. The relationship between GA and ABA was summarized by Ogawa et al. (2003) in three possible ways: 1) GA reduces ABA levels by affecting ABA biosynthesis; 2) GA negatively regulates the ABA response pathway; or 3) GA and ABA signals are targeted independently to distinct cis-regulatory sequences of a single gene. On this basis, it is apparent that the GA and ABA relationship in sandalwood seeds follows the third suggested way, where their signals are targeted independently. In other plant species such as wheat and rice, ABA–GA balance was found to be the major regulator of seed dormancy and germination with high GA content leading to decreased dormancy (Castro-Camba et al. 2022). GA_3 treatment has been shown to reduce endogenous ABA levels in germinating seeds of lettuce (Toyomasu et al. 1994), confirming our findings in which ABA levels in GA-treated seeds did not change significantly.

Our findings have practical implications for enhancing the cultivation of sandalwood. GA3 has been proven to be effective and can improve germination rates, eliminating the need for stratification or other less effective methods. This advancement in propagation supports the sustainable production of sandalwood, making it a more widely used means of combating desertification, increasing biodiversity, and producing economic benefits. Future research should focus on improving GA3 concentrations and exploring the long-term effects of its application on seedling growth and development. In addition, research into which and how the sandalwood is undergoing dormancy will provide more information

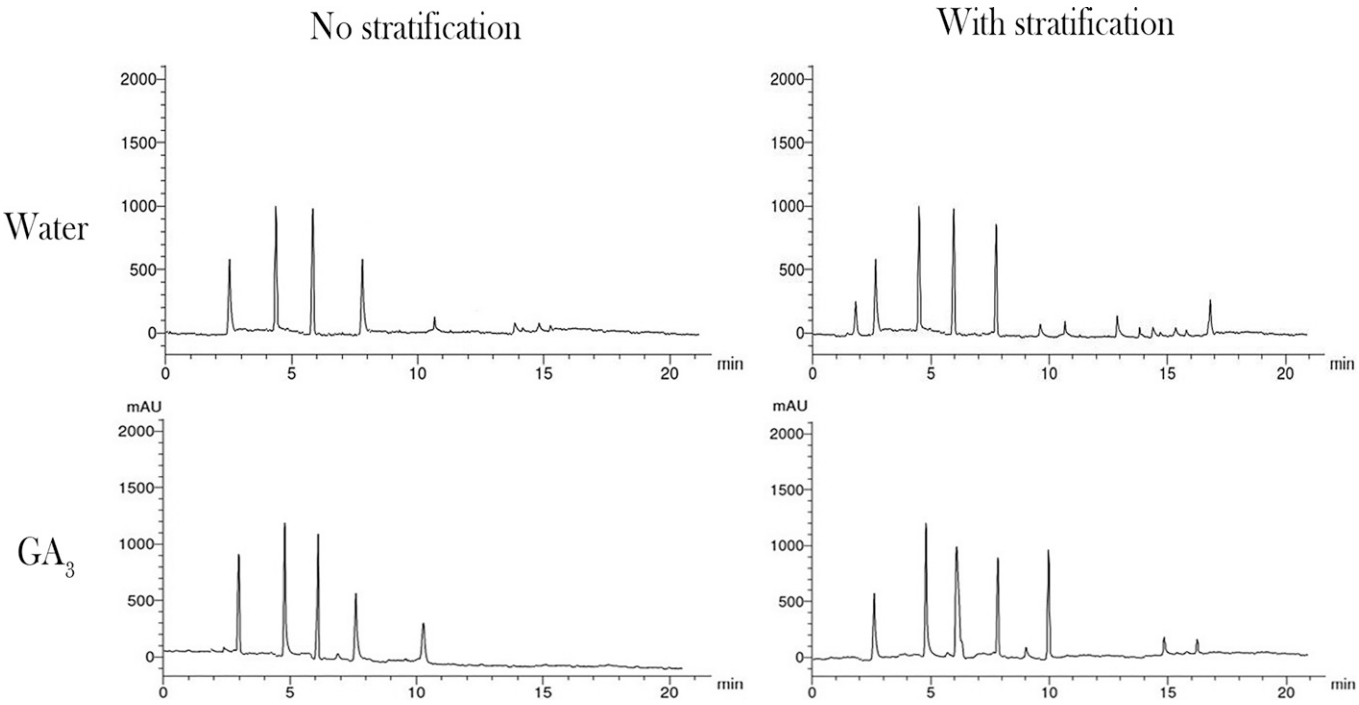

Fig. 3. Representative high-performance liquid chromatography chromatograms of sandalwood seeds treated with pre-germination soaking in water or gibberellic acid (GA_3) with/without stratification.

about the requirements for its successful propagation; this could lead to more efficient and sustainable practices in planting and handling.

Conclusions

The outcomes indicate that GA_3 treatment greatly improves the rate and percentage of germination in sandalwood seeds. In contrast, stratification lowered germination rates, possibly because it reduced phytohormone levels. HPLC analysis showed that GA_3-treated seeds had higher levels of germination-promoting hormones (GA_3, IAA, and BAP), but their concentrations were reduced by stratification, especially in GA_3. Other factors may have played a role in reduced germination under stratification because both treatments and ABA levels did not differ.

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