The influence of scarification and media containing vesicular arbuscular mycorrhiza on germination of sandalwood (Santalum album Linn) seeds

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Abstract. Sandalwood (Santalum album) is an essential species as containing fragrant substances in the heartwood. Seed germination is one of the essential keys to the success of sandalwood development, and the use of vesicular-arbuscular mycorrhiza (VAM) is also essential to plant enhancement. This study aimed to obtain information on the influence of scarification techniques and the use of VAM on germination media to sandalwood seed germination. Five scarification treatments (control, soaked in water for 24 and 48 hours, GA₃ 300 and 500 ppm for 17 hours) and two VAM application (with and without VAM) were employed (split-plot with completely randomized design (CRD)). The result showed that the cumulative germination was higher on the seed with scarification of soaked in GA₃ solution 300 and 500 ppm than control, soaked in water 24 H and 48 H. This pattern also appeared on germination index. Mean germination time showed slower on control, soaked in water 24 H and 48 H than the use of GA₃ 300 and 500 ppm. The use of VAM did not significantly affect the cumulative germination, germination index, and mean germination time. A time-series observation showed that seed sown on media with VAM tended to germinate slower than media without VAM.

1. Introduction
Sandalwood is an economically important species. S. album, naturally, contains a fragrant substance in the heartwood that is used to manufacture products with many purposes including religious, medicinal, in perfumery and cultural ceremonies [1]. As the high value of sandalwood, the native population of S. album has experienced extensive exploitation for commercial purposes which has caused numbers of trees to decrease in nature [2]. In 1996, an assessment of S. album conservation status, through an Asian regional workshop in Viet Nam, declared the tree as a vulnerable species due to this extensive exploitation; and International Union published this assessment for Conservation of Nature and Natural Resources (IUCN) [3] in 1998.

Given the poor condition of sandalwood (S. album) population and the decline on supply, many countries are establishing sandalwood plantation tropical regions including India, Sri Lanka, Australia, China and Fiji [4]. This condition has also attracted scientists to research sandalwood development across aspects has been studied including sandalwood silviculture [5], growth responses after VAM
application on sandalwood seedlings [6] and the use of different container sizes on S. album growth performance [7].

Seed germination in sandalwood is an essential aspect in terms of sandalwood development because the quality of germination can be affected by provenance, mother tree, or pre-treatment. Wawo [8] showed that seed from different mother trees and provenances had an effect on the seed germination successful. The lowest germination could be only 1.7 % from 990 seeds, and the highest was +achieved 87.93 % from 630 seeds. Other studies showed that sandalwood seed without treatment showed less germination rate compared to those with pre-treatment [9, 10].

There were studies elucidated that the use of VAM could also enhance the performance of seed germination on other species. A study by Ghimire et al. [11] found that mycorrhizal fungus has enhanced the seed germination of Panicum virgatum (Switchgrass), this has a similar result on a crop species that mycorrhiza has increased germination rate of vanilla [12]. Nevertheless, other studies showed a different pattern that the presence of mycorrhiza had declined the germination rate of Geranium sylvaticum but did not affect the performance of the seedlings [13]. In terms of sandalwood seed, little is known on how the seed scarification and the use of VAM on media influence the germination of sandalwood seed.

This paper reports a study on sandalwood seed germination under factorial experiment to obtain information on the quality of seed germination of sandalwood after receiving treatments of five seed scarification under media containing VAM.

2. Method

2.1. Materials
The sandalwood seeds used in this study were obtained from the University of The Sunshine Coast, Queensland, Australia. The mycorrhiza used was a commercial product produced by Mycorrhizal Applications International (Australia) Pty Ltd, Western Australia. The product contained 220,000 propagules/kg with four species of VAM which were Glomus aggregatum, G. etunicatum, G. intraradices and D. mosseae.

The media used for seed germination consisted of sand and vermiculite with a ratio of 1:2 (v/v). The media was then combined and sterilized for 3 hours at 100°C using oven-dry sterilization in which the media was stirred every hour; thus, the media could be evenly sterilized. The sterilized media was then placed on ten trays with 3 L of volume, and each tray received 50 g of VAM.

2.2. Procedure
This study was undertaken under greenhouse condition from October 2017 to January 2018 at Southern Cross University in Lismore, New South Wales. The maximum temperatures were 34 – 50°C and minimum temperatures were 22 – 24°C with relative humidity between 34 – 74 %. This experiment was arranged in a split-plot with completely randomized design (CRD).

Two factors of factorial treatment were the use of mycorrhiza (non-mycorrhizal media and mycorrhizal media) and seed scarification. Seed scarification was control (directly sown on media), soaked in water for 24 hours and 48 hours, and soaked in gibberellic acid (GA₃) of 300 and 500 ppm for 17 hours. The treatment combinations were replicated four times with each replication used 25 sandalwood seeds; thus, 1,000 seeds in total were employed. Seeds at each repetition were arranged at a 5 x 5 grid to identify the germination. All sandalwood seeds were sown on 5th October 2017.

2.3. Measurement and analysis
Cumulative germination (percentage) (CG) was measured every five days starting from first emerging germinated seed, where at the final measurement all germinated seeds were divided by the total of seed at the same treatment. Mean germination time (MGT) was determined as described in Hartmann and Kester [14] and germination index (GI) was determined as described in Dezfuli et al. [15]. The percentage of data of CG was arcsine transformed before the ANOVA analysis. Any significant result
from ANOVA was then analyzed using Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$ using SPSS version 23.

3. Result and discussion

The study used five different seed treatments on *S. album* seed significantly influenced CG, GI, and MGT, as presented in table 1. The use of VAM on media not significantly influenced CG, GI, and MGT. Interaction between scarification techniques and the use of VAM on media only appeared on MGT.

**Table 1** Analysis of variance on the application of scarification and the use of VAM

| Source            | df  | CG (Sig. value) | GI (Sig. value) | MGT (Sig. value) |
|-------------------|-----|-----------------|-----------------|------------------|
| Mycorrhiza (M)    | 1   | 0.330           | 0.116           | 0.128            |
| Scarification (S) | 4   | 0.000*          | 0.000*          | 0.001*           |
| M x S             | 4   | 0.242           | 0.168           | 0.023*           |

* is significant difference at $\alpha = 0.05$

3.1 Scarification techniques effects

The results of scarification techniques on GI, MGT, and CG are summarised in table 2. Scarification techniques with GA3 both 300 ppm and 500 ppm have significantly enhanced the quality of sandalwood seed germination compared to other scarification such as control, soaking in water for 24 and 48 hours after three months of the experiment. The use of GA3 had influenced GI both on 300 and 500 ppm (4.13 and 4.11) greater than control, soaked in water for 24 and 48 hours, which was 0.28, 0.3 and 0.24 respectively. MGT showed the best result on GA3 both 300 and 500 ppm at 40.20 and 43.34 days compared to control, soaking in water for 24 and 48 hours (65.82, 72.85 and 55.97 days). The use of GA3 had increased cumulative germination (300 ppm = 74.5 % and 500 ppm = 78 %) whilst control, soaking in water 24 and 48 hours were at 10.5 %, 14 % and 9.5 % each.

**Table 2** Average value of scarification treatment on GI, MGT, and CG

| Scarification Treatment | GI   | MGT (day) | CG (%) |
|-------------------------|------|-----------|--------|
| Control                 | 0.28 a | 65.82 bc  | 10.5 a |
| Soaked in water 24 H    | 0.30 a | 72.85 c   | 14 a   |
| Soaked in water 48 H    | 0.24 a | 55.97 ab  | 9.5 a  |
| GA3 300 ppm             | 4.13 b | 40.20 a   | 74.5 b |
| GA3 500 ppm             | 4.11 b | 43.34 a   | 78 b   |

Marked with similar letters is not significantly different at $\alpha = 0.05$ using DMRT analysis

3.2 The use of VAM on media

The presence of VAM on media on GI, MGT, and CG is presented in table 3. Even though the results showed not significantly different, the pattern of the use of VAM on media can be seen. The GI of media without VAS was more significant than with VAM (1.98 and 1.65 respectively). CG on media without VAM also showed a similar pattern that was greater than with VAM (39 % and 35.6 % each). Meanwhile, MGT on media with VAM showed faster than without VAM, which were 51.64 days and 59.64 days, respectively.
Table 3 Average value of media treatment on GI, MGT, and CG

| Media Treatment | GI  | MGT (day) | CG (%) |
|-----------------|-----|-----------|--------|
| Without VAM     | 1.98 a | 59.64 a  | 39 a   |
| With VAM        | 1.65 a | 51.64 a  | 35.6 a |

Marked with similar letters is not significantly different at $\alpha 0.05$ using DMRT analysis

3.3. Time-series observation on sandalwood seed germination

A full-time observation was undertaken to obtain information on germination trend of sandalwood seed, and the results are presented as a line graph in figure 1. The use of $\text{GA}_3$ both 300 ppm and 500 ppm showed faster first germination (day 15) compared to other scarification techniques (day 35). Meanwhile, the use of VAM on media showed patterns that the addition of VAM on media for germination tended to slow the germination on same scarification methods. The seeds under $\text{GA}_3 300$ ppm on media without VAM had a more significant number of germinated seeds (A1) at day 22 (7 seeds) compared to media with VAM (1 seed). This pattern continued to day 39 that media without VAM resulted 15 seeds compared to media with VAM at 13 seeds. This pattern also appeared on other scarification treatment of $\text{GA}_3 500$ ppm (B1), control (E1), and soaking in water 48 hours (D1). Nevertheless,
This study aimed to enhance the germination of sandalwood seeds under the combination of scarification techniques and the use of VAM. Sandalwood seeds are low on germination without pre-treatment of scarification (control) as shown in this study. The use of water for pre-treatment also showed low germination. The imbibed seed after soaked in water for 24 and 48 hours may not be able to stimulate the germination. The previous study has also shown that sandalwood seeds without pre-treatment or soaking in water have low cumulative germination that affects mean germination time because the time to germinate between seeds is prolonged [16]. Soaking seeds in a solution of GA$_3$ had enhanced the performance of germination on sandalwood seeds. GA$_3$ may stimulate the hydrolytic enzyme activity that is known induced by GA$_3$ [17]. The use of GA$_3$ has been successful in improving the germination performance at a variety of species [18, 19]. However, the use of scarification

![Graphs of germination](image)

**Figure 1.** Graphs of a full-time observation on sandalwood seed germination. The left-hand side graphs (A1, B1, C1, D1, E1) are the number of germinated seeds every five days, the right-hand graphs (A2, B2, C2, D2, E2) are the CG in percent measured every five days. The **black circle** is media without mycorrhiza, and the **white square** is media with mycorrhiza.
techniques combined with the use of VAM on media for germination has indicated to declining performance of sandalwood germination.

VAM is commonly associated with plant roots in order to enhance plant growth and on land remediation through plant-VAM association [20]. The use of VAM in this study could be a new insight as little is known on the influence of the use of VAM on media for germinating sandalwood seeds. Studies on the use of mycorrhiza affecting seed germination have been examined with different results. The earliest study by Sreedhar and Mohan [19] on woody species (Gmelina arborea, Alianthus excelsa, Melia dubia, and Neolamarckia cadamba) showed that the use of VAM on germination media has improved germination, but a study by Varga [13] showed inhibition on germination of Geranium sylvaticum. Even though the result of GI and CG is not significant statistically, the pattern may be seen that media with VAM tended to have low GI and CG. GI is one parameter that can be used to describe which types of germination media give the best rates of germination because this index combines an assessment of the speed of germination and cumulative germination [21].

The inhibition pattern on germination as the result of the use of mycorrhiza in the sandalwood seed germination could also be seen in the fulltime-observation, as shown in figure 1. Only in the seed treatment with seeds soaked in water for 24 hours had a different pattern, in which the use of VAM appeared to have a better result than without VAM. However, the other seed treatments showed a similar pattern in which the use of VAM on the germination media negatively affected seed germination. In the seed treatment with GA3 300 and 500 ppm, for example, in terms of the time need for germination, the presence of mycorrhiza in a media tended to slow the germination, although the final cumulative germination was similar within seed treatment on GA3 500 ppm. The use of VAM also negatively affected the final germination of the three seed treatments (control and soaking seeds in the water for 48 H). These indicated that the use of mycorrhiza might affect the speed and cumulative germination of sandalwood seed.

Conclusion
The presence of mycorrhiza in media for germination did not accelerate the speed of germination. Even though the results are not significant statistically, the pattern showed that VAM on media seems to decline germination index and cumulative germination. Moreover, the time series observation revealed that there was a pattern on S. album seed germination in media without mycorrhiza have faster seed germination compared to media with mycorrhiza.

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