

***In-vitro* germination of red sandalwood (*Pterocarpus santalinus* L.) seeds**

G.D.G. Chaturani, M.P. Jayathilleke and S. Subasinghe

Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Kamburupitiya.

Abstract

*Red sandalwood (*Pterocarpus santalinus* L.) is a valuable medicinal plant, which is included in the red list of endangered plant species. Rapid in-vitro propagation system for the conservation of the plant, is becoming important as conventional methods are not satisfactory. For this purpose the feasibility of in-vitro establishment of red sandalwood seeds was tested. Seeds were treated with mercuric chloride (0.1 %) for different durations to select a suitable surface sterilization procedure. Pods with external diameter of < 3, 3-4, 4-5, and > 5 cm were used to examine the effect of the size of pods on in-vitro germination. Pods harvested at light brown stage and stored at ambient temperature (28 ± 2 °C) for 1, 2, 3, and 4 week(s) were used to examine the effect of storage time on in-vitro germination. Treating seeds with 0.1 % HgCl₂ (with 2-3 drops of Tween 20) for fifteen minutes was effective for surface sterilization of seeds. Pods with < 3 cm of diameter contained no viable seeds. Seeds obtained from pods having a diameter of > 4 cm showed a significantly higher germination (90 %) ability while time taken for germination was not affected by pod diameter. Successful seed germination reduced with increasing storage time. Seeds cultured after one week of storage showed the highest rate of germination (96 %) and shortest germination time (8 days), while seeds cultured after 4 weeks of storage showed a lower rate of germination (61 %) and longer germination time (10 days).*

Key words: Germination, In-vitro, Red sandalwood, Seeds, Storage

Introduction

Red Sandalwood (*Pterocarpus santalinus* L.) belonging to the Family Fabaceae is an evergreen tree species, and is chiefly used as an Ayurvedic medicine. *P. Santalinus* shows a very low fruit set of about 6 % and out of that, seed set is only 52% (Rao and Raju, 2002) and propagation of the plant through seed is very much limited as the pod contains a very hard seed coat. The pods would not germinate unless its hard coat is scarified manually, and hence pods can be found lying under the tree for a very long time without germination. Soaking and drying of scarified pods was found to be successful in seed propagation up to 48.8 % only (Kumarasinghe *et al.*, 2003). Earlier studies using conventional propagation methods like grafting and rooted cuttings have not been very successful (Sita *et al.* 1992). Hence an alternative rapid propagation system is acutely needed to conserve this endangered medicinal plant. Thus the major objectives of this research were to study the relationship of pod size and storage time on *in-vitro* seed germination rate and to successfully establish Red Sandalwood seeds in culture.

Materials and Methods

Pods of Red Sandalwood (*Pterocarpus santalinus* L.) turning into brownish color were picked up from selected trees and used as initial plant material for the experiments. Experiments were designed according to Complete Randomized Design. Data were analyzed using SAS statistical software and Duncan's Multiple Range Test was used for mean separation.

Experiment 01: Selection of suitable surface sterilization procedure for the establishment of red sandalwood seeds

Seeds were extracted from the pods and the undamaged seeds were thoroughly washed in a detergent solution (5% Teepol) and kept under running tap water for 2 hours. The seeds were then shaken in a solution of 0.1% HgCl₂ with 2 drops of Tween 20 for 5, 10, 15, and 25 min followed by rinsing in sterile distilled water for 4 times. The sterilized seeds were cultured on full strength hormone free MS medium, with 10 replicates. Contamination rate and germination percentage were recorded.

Experiment 02: Effect of the pod size on in-vitro germination of red sandalwood seeds

Collected pods were categorized into four groups according to their external diameter (< 3 cm, 3-4 cm, 4-5 cm, and >5 cm) (Plate 01). Seeds were extracted from pods without damage. Then the seeds were washed in a detergent solution (5% Teepol) and kept under running tap water for 2 hours prior to surface sterilization using 0.1 % HgCl₂ for 15 min. Sterilized seeds were cultured on full strength, hormone-free MS medium. Each treatment consisted of 10 replicates. Time taken for germination and percentage of germinated seeds were recorded.

Experiment 03: Selection of suitable surface sterilization procedure for the establishment of red sandalwood seeds

Seeds were extracted from the pods carefully without damage. Undamaged seeds were selected and thoroughly washed with Teepol (5%) and kept under running tap water for 2 hrs. Then seeds were dipped in 0.1% HgCl₂ with two drops of Tween 20 for 5, 10, 15, and 25 minutes and shaken continuously throughout the time. Sterilized seeds were cultured on full strength hormone free MS medium, with 10 replicates. Contamination and germination percentage were recorded.

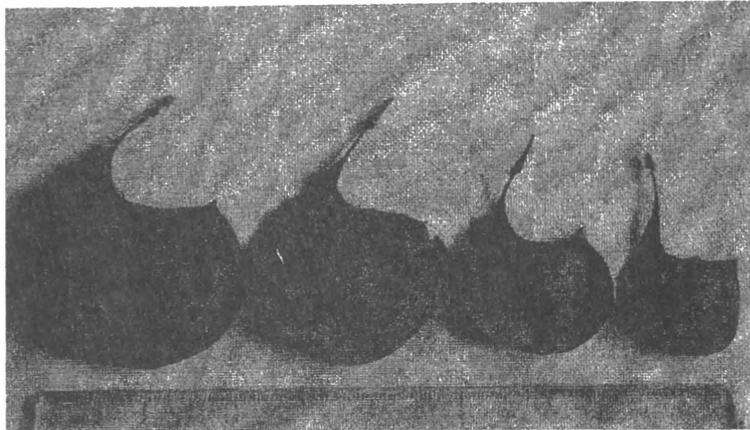


Plate 01: Categorization of Red Sandalwood pods according to the external diameter

Experiment 04: Effect of storage time of the pods on in-vitro germination of red sandalwood seeds

Pods with external diameter more than 4 cm were stored at ambient temperature (28 ±2 °C) for one, two, three, and four weeks. At the end of storage period seeds were excised from the pods carefully. Seeds were then surface sterilized using 0.1 % HgCl₂ for 15 min and cultured on full strength, hormone-free MS medium, with 10 replicates. Time taken for the germination and germination percentage were recorded.

Results and discussion

Treating the seeds with 0.1% HgCl₂ for 15 min was found to be effective in surface sterilization of seeds. The contamination rate in this treatment was less than 10 % (Figure 05). When the exposure time to HgCl₂ was less than 15 min, a very high rate of contamination was observed. When the exposure time was increased up to 20, 25 min, the contamination rate was found to be very low. Seed Germination also reduced significantly due to fungal and bacteria contaminations with lower exposure time to HgCl₂ (Figure 06). *In-vitro* germination of red sandalwood seeds was significantly increased with increasing pod diameter. No viable seeds were available in pods less than 3 cm in diameter and therefore no germination was observed. Pods having a diameter above 3 cm contained viable seeds. Seeds obtained from pods having a diameter of above 4 cm showed a significantly higher (90 %) germination ability (Figure 01) whereas seeds of pods with 3-4 cm diameter showed less germination (42 %) ability. However, there was no significant effect of pod diameter on time taken for seed germination (Figure 02). Some pods (0.1 %) with a diameter of more than 5 cm contained two seeds instead of one. Such pods should be discarded as the seed germination is poor due to their small size. Seeds with abnormal morphology or very dark brown testa should also be discarded as they do not germinate into plantlets.

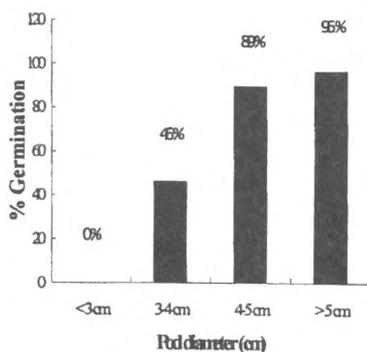


Fig 01: Effect of pod diameter on *in-vitro* germination

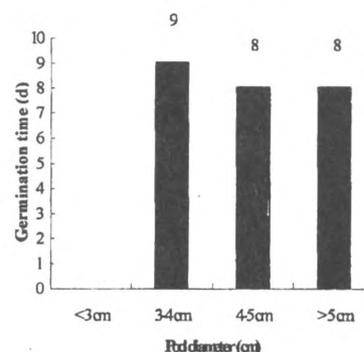


Fig 02: Effect of pod diameter on *in-vitro* germination

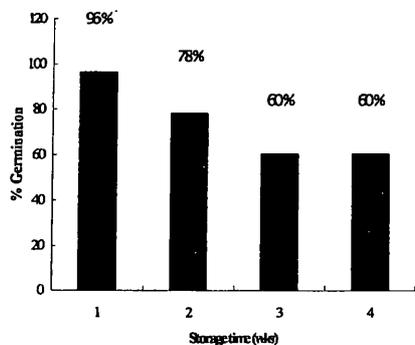


Fig 03: Effect of storage period on *in-vitro* germination

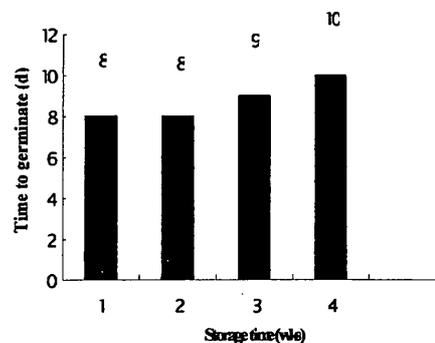


Fig 04: Effect of storage period on *in-vitro* germination time

Storage time of pods also showed a significant effect on in-vitro germination of seeds. Seed germination decreased with increasing period of storage. The highest germination (96 %) was recorded in seeds extracted from the pods, which were stored only for one week (Figure 03). When pods stored for a longer period of time, germination ability gradually declined. Seed germination was only 60 % when pods were stored for three weeks. Time taken for seed germination also increased significantly with increasing storage time (Figure 04). When pods were stored for only one week, seed germination was observed within 8 days and when the storage period was increased up to four weeks, it took more than 10 days for the seeds to germinate.

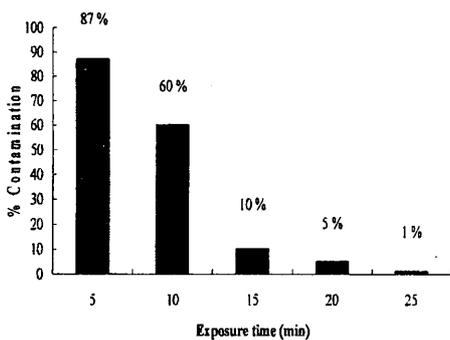


Fig 05: Effect of exposure time in 0.1% HgCl₂ on contamination percentage

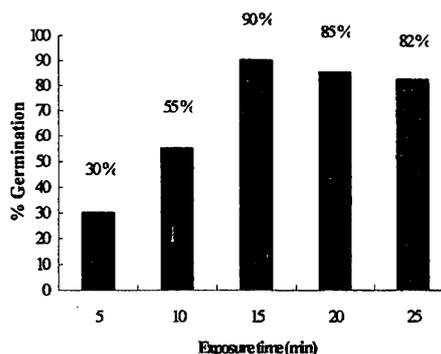


Fig 06: Effect of exposure time in 0.1% HgCl₂ on in-vitro germination %

Conclusions

1. Seeds should not be stored for more than one week to ensure higher germination.
2. Seed germination was significantly high in pods having a diameter of more than 4 cm whereas, poor seed germination was observed in pods of < 4 cm diameter.
3. Treatment of seeds in 0.1 % HgCl₂ (with two drops of Tween 20) for 15 minutes was effective for surface sterilization of seeds.

References

Rao, S. P. and Raju, A. J. S. (2002). Pollination Ecology of the Red Sandawood *Pterocarpus santalinus* (Fabaceae), an endemic and endangered tree species. *Current Science*. 83(9): 1144-1148.

Sita, G. L., Sreenatha, K. S. and Sujatha, S. (1992). Plantlet production from shoot tip culture of red sandalwood (*Pterocarpus santalinus* L.). *Current Science*. 62(7): 532-535.

Kumarasinghe, H. K. M. S., Subasinghe, S., Arunakumara, K. K. I. U. (2003). Studies on seed propagation of Rathhadun (*Pterocarpus santalinus* Linn.) as affected by method of soaking and scarification. *Proceedings of 9th Annual Forestry and Environment Symposium*. 57p