OriginalArticle

In Vitro Seed Germination and Optimization for Dalbergia Sissoo Roxb

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ABSTRACT
Dalbergia sissoo, a commercially significant legume, faces challenges in propagation and cultivation due to factors such as salinity. This study aimed to optimize seed germination conditions for this species. Seeds were subjected to various sterilization methods and germinated on filter paper, semi-solid, and liquid MS media. Results indicated that liquid MS medium exhibited the highest germination rate (67.53%), followed by semi-solid MS medium (57.14%) and filter paper (47%). These findings suggest that liquid MS medium provides an optimal environment for Dalbergia sissoo seed germination. Further research is necessary to evaluate the impact of salt stress on germination and to explore in vitro propagation techniques for this valuable species.

Keywords: Propagation, Germination, MS medium, Dalbergia sissoo, Valuable

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Introduction

The genus *Dalbergia* is part of the legume family Fabaceae and the subfamily Papilionoideae. This tropical genus includes around 100 species of trees, shrubs, and lianas. One species, *Dalbergia sissoo*, is a medium to large tree that can reach approximately 25 meters in height, with a grey-yellow trunk measuring 2-3 meters in diameter. Its leaves are leathery and pinnately compound, with alternate, broad, ovate, acuminate, glabrescent leaflets that have a fine-pointed tip [(1)]. *Dalbergia sissoo* primarily reproduces by seed, allowing it to form dense thickets. The flowering season is from March to May. It is found in an exotic range including Bangladesh, Bhutan, India, Malaysia, and Pakistan, and a native range covering Cameroon, Ethiopia, Indonesia, Iraq, Israel, Kenya, Mauritius, Nigeria, Sudan, Tanzania, Thailand, Togo, the US, and Zimbabwe [(2)].

Dalbergia sissoo is renowned for its medicinal properties and has been traditionally used to treat various ailments including skin and blood diseases, syphilis, stomach issues, dysentery, nausea, and disorders of the eye and nose[(3)]. It also serves as an aphrodisiac and expectorant. Indian rosewood exhibits insecticidal and larvicidal properties and is resistant to some wood-boring insects[(4)].

Plant cell, tissue, and organ cultures leverage the "totipotency" of plant cells, which allows them to regenerate into a complete organism. This technique enables the growth of isolated cells, tissues, differentiated organs, or whole plants in a growth medium under sterile conditions, outside their natural environment [(5)].

The following study was carried out with the following objectives as to assess the seed viability of *Dalbergia sissoo* Roxb. seed germination percentages were determined using the filter paper method conducted in liquid and semi-solid basal MS media, with varying durations of sterilization and to evaluate seed germination percentage and viability under salt stress, seeds were subjected to different concentrations of Sodium chloride (NaCl) in basal MS medium.

Material And Methodology

Following methodology was conducted to check the seed germination percentage by cultivating seeds on filter paper and in liquid and semi-solid basal MS media.

Sample (seeds) Collection

Seeds of *Dalbergia sissoo* Roxb were obtained from Forest Research Institute and experiment was performed at Graphic Era University, Dehradun. These seeds were utilized as the sample material for the experiments.

Preparation of filter paper medium and basal liquid and semi-solid MS media

Sterile and autoclaved petri plates were supplied with Whatman filter paper serving as a platform for germination of seeds. Total 10 petri plates were prepared for the purpose. Total 7 test-tubes containing semi-solid basal MS medium were utilized for seed germination. Following this, 7 test tubes containing

liquid basal MS medium supplied with filter-paper bridge acting as platform for seeds germination.

Seeds surface sterilization treatment

Seeds of *Dalbergia sissoo* soaked in a solution of liquid detergent (Tween-20, 2-3 drops) for 10 minutes and 0.1%, w/v) for a time period of 10 minutes rinsed with autoclaved distilled water. To assess the viability and germination percentage rate of the *Dalbergia sissoo* seeds, ten petri plates each with ten seeds were cultivated on moist filter paper and seeds were also inoculated in test tubes containing semi-solid basal MS medium (two bunches of seven test tubes each).

Seed Viability Test and Germination percentage on basal liquid MS medium

Using liquid basal medium supplied with a filter paper bridge, to measure the germination percentage and viability of *Dalbergia sissoo* seeds

Surface Sterilization of Seeds of *Dalbergia sissoo*

Dalbergia sissoo seeds were treated with liquid detergent solution (Citrimide, 4-5 drops) for a time period of 10 minutes. Following this, seeds were treated with an aqueous solution of the fungicides Dithane M-45 (0.1%, w/v) and Bavistin (0.2%, w/v) for 15 minutes in order to decrease contamination. Seeds were surface sterilised for 7 minutes using Mercuric chloride (Hgcl2, 0.1%, w/v) before inoculation. Single seeds were inoculated in liquid basal MS media in a test tube total 11 sets (one set has 7 test tubes).

Results and Discussion

In vitro seed germination percentage of *Dalbergia sissoo* seeds on filter paper

Ten petri plates with moist filter paper were utilized to grow seeds of *Dalbergia sissoo.* Each petri plate consists of 10 seeds **(Figure 1)**. The observations and calculations were done after 30 days of induction of cultures **(Table 1)**, **(Figure 2)**. A graph was plotted between number of replicates and number of seeds was germinated on filter paper **(Graph 1)**.The seeds showed 47% of germination rate. It means that atleast 50 percent seeds are in viable condition and able to germinate on moist filter paper.

***In vitro* seed germination of *Dalbergia sissoo* seeds grown on semi-solid basal MS medium**

To check seed viability and germination percentage, 14 test tubes supplemented with semi-solid basal MS media were utilized for inoculation of sterilized seeds. Each test tube consists of only 1 seed **(Figure 3).** After rinsed with distilled water. Pre-disinfection treatments of thefungicide Bavistin (0.2%, w/v) and Dithane M-45 (0.1%, w/v) for duration of 15 minutes. Following these steps, seeds underwent surface sterilization using Mercuric chloride (HgCl2,

30 days of culture induction, number of seeds germinated and their germination percentage was calculated **(Table 2), (Figure 4).**Overall seed germination percentage was calculated as 57.14%.

***In Vitro* seed germination of *Dalbergia sissoo* seeds grown in liquid basal MS medium**

Total 11 sets of test tubes **(one set consists of 7 test tubes)** were prepared which consists of basal liquid MS medium and filter-paper bridge which provides a platform for germination **(Figure 5)**. Observations and calculations were recorded after 15 days of induction **(Figure 6, Table 3)**. Only 55 seeds out of 77 were shown good germination with better growth. A graph was plotted between number of test tube sets containing seeds in basal liquid MS medium and number of seeds germinated in each set (**Graph 2)**. Overall seed germination percentage of 77 seeds was calculated as 67.53 %.

Conclusion

Dalbergia sissoo, a commercially important legume, faces challenges due to salinity and propagation difficulties. This study aimed to optimize seed germination conditions for this species.Results indicated that **filter paper**and **semi-solid MS medium** supported seed germination, with **47% and 57.14% germination rates,** respectively. However, **liquid MS medium** demonstrated the highest germination rate of **67.53%.** These findings suggest that liquid MS medium provides a suitable environment for *Dalbergia sissoo* seed germination.

Further research is warranted to investigate the influence of different salt concentrations on seed germination and seedling growth, as well as to explore the potential of in vitro techniques for large-scale propagation and salinity tolerance improvement in Dalbergia sissoo.By optimizing germination protocols and understanding the effects of salinity, this study contributes to the development of sustainable cultivation practices for this valuable species.

Conflict of Interest- There is no conflict of interest among the authors.

1. **TABLES**

Table 1- Table shows number of petri plates utilized, number of seeds germinated in each petri plates out of 10 seeds and the overall germination percentage of all 100 seeds.

Number of Replicates of seeds grown on filter paper	Number of seeds germinate (Out of 10)	Overall Germination Percentage (Considered of all 10 replicates)
I	4	**47/100*100 = 47%**
II	5	
III	5	
IV	7	
V	0	
VI	5	
VII	6	
VIII	5	
IX	6	

X	4	

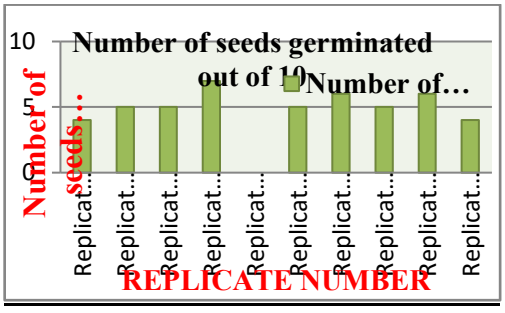
Table 2- Germination percentage of seeds grown on semi-solid MS medium after 30 days.

Number of test tubes containing	Observation of seed germination	Overall Germination Percentage
a.	No seed germinated	
b.	No seed germinated	
c.	Seed germinated	
d.	Seed germinated	
e.	Seed germinated (Plantlet with two leafs)	
f.	Seed germinated (Plantlet with five leafs)	**8/14*100 = 57.14%**
g.	Seed germinated	
h.	Seed germinated (Plantlet with 7 leafs)	
i.	No germination	
j.	No germination	
k.	Seed germinate (Plantlet with nine leafs)	
l.	No germination (Fungal contamination)	
m.	Seed germinate	
n.	No germination	

Table 3- Germination percentage of seeds grown on liquid MS medium after 15 days.

Number of test tube sets	Seed Germinate (out of 7)	Overall Percentage
I	5	
II	6	
III	7	
IV	4	
V	5	
VI	6	
VII	6	**52/77*100 = 67.53%**
VIII	4	
IX	7	
X	5	
XI	4	

FIGURES



Graph 1: Graphical representation between Replicate numbers and number of seeds (out of 10) germinated in each replicate.

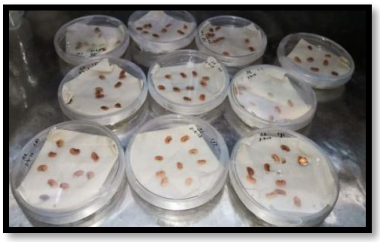
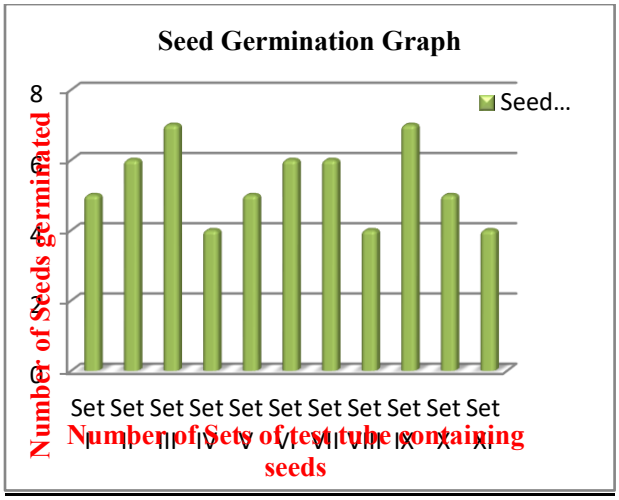


Figure 1: 10 petri plates containing *Dalbergia sissoo* seeds (each petri plate contains 10 seeds) growing on moist filter paper (at inoculation time).

Figure 2: Seeds germination on filter paper, observations after 30 days of induction.

Figure 3: Dalbergia sissoo seeds inoculated on semi-solid basal MS media.

Figure 4: Seeds germination on semi-solid basal MS media, observations after 30 days of induction.



Graph 2: Graphical representation between number of test tubes sets containing seeds and number of seeds germinated.

Figure 5: Dalbergia sissoo seeds inoculated on liquid basal MS media. Images were taken after 7 days of inoculation.

Figure 6: Dalbergia sissoo seeds germinated on liquid basal MS media after 15 days of inoculation.

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