Catalyzing Germination: Exploring Scarification and Hot Water Treatments for Dormancy Breaking and Enhanced Storage in *Ficus Benjamina* L., *Thespesia populnea* L., *Phyllanthus emblica* L., *Tectona grandis* L.f. seeds

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Abstract

A seed is a significant stage in the plant life cycle and is often referred to as the dispersal unit of the plant. There are two types of seeds: dormant seeds and non-dormant seeds. A dormant seed is one that does not have the capacity to germinate within a definite period under any combination of normal, physical and environmental factors. The other type is favourable for germination and when the seed becomes non-dormant, the circumstances that break dormancy and the location of water gaps in seeds, remain unclear.

In the present study, we consider the adaptive role of impermeable coats in the seeds of Ficus Benjamina L., Thespesia populnea L., Phyllanthus emblica L. and Tectona grandis L.f. The study was particularly designed to analyze the conditions that break dormancy and the location of the primary water gap during dormancy breaking. Each seed was treated separately to break its dormancy and it was observed that water entered only through the lens due to the dislodgement of the palisade layer. Additionally, the storage life of Ficus Benjamina L. and Phyllanthus emblica L., seeds was extended as evidenced by the analysis of low moisture content decreasing from 6.02% to 5.31%. This study concludes that the impermeable seed coat of seeds with low moisture content increases storage life and regulates seed imbibition, thus influencing germination with the growing season.

Keywords: Imbibition, Moisture content, Water gap, Physical dormancy, Storage life.

Introduction

Seed dormancy is a significant phenomenon in which intact viable seeds are unable to complete germination under favourable environmental conditions and these seeds are referred to as dormant seeds.

On the other hand, seeds that are capable of germinating under any condition are known as non-dormant seeds[17]. There are five different types of dormancy: physiological (PD), morphological (MD), physical (PY), morphophysiological (MPD) and combinational dormancy (PY + PD)[7].

Various techniques are commonly employed to enhance the germination of dormant seeds. These methods include pre-chilling, scarification, nitric acid treatments and the use of various growth regulators such as Gibberellins, Cytokinin and Ethylene[8]. Both mechanical and chemical scarification treatments have been found to have a relative effect in breaking the physical dormancy of seeds in species like *Bituminaria basaltica*, *Ormosia arborea* and *Luffa cylindrica*[5].

Exogenous application of gibberellic acid has been shown to effectively overcome seed dormancy in several species including *Rheum webbianum*, *Carum carvi*, *Saussurea lappa* and *Bunium persicum*[23]. Another active method for breaking dormancy in seeds involves the application of cold stratification and gibberellic acid as demonstrated in species like Ducrosia *Anethifolia* and *Brassica tournefortii*[6].

The formation of a water gap is irreversible and directly influences the timing of seed germination[15]. Abundant and detailed studies have been conducted to identify water gaps in families such as Phyllanthaceae, Moraceae, Lamiaceae, Malvaceae. Once the water gap is opened, the structures cannot close again and germination usually commences immediately if suitable environmental conditions persist, except for seeds with combinational dormancy (PD + PY).

The primary and secondary water gaps in seeds with physical dormancy are collectively termed the 'water gap complex' and the term 'water gap 'is used to describe the primary opening in the seed coat during dormancy break. Three types of water gaps are recognized: the lens, hilar slit and micropyle[13]. The lens is a specialized site for water uptake, in addition to the hilum and micropyle, which are also important for imbibition in seeds of some species[9]. In some cases, the lens present in the seed coat acts as a water gap whereas in some species, structures other than a lens function as a water gap[13].

Water gap complexes can be categorized into three types based on the type of openings they create: type I, water gaps with narrow linear openings occluded by modified elongated

palisade cells; type II, water gaps formed as circular or narrow openings occluded by lid-like structures resulting from palisade cells and type III, water gaps with either narrow or circular openings occluded by plug-like structures formed by sclerenchyma cells.

In Fabaceae members, the lens differs in location, morphology and anatomy within the family or between genera of the same subfamily[18]. In Moraceae members, the water gap is a small plug in the seed coat adjacent to the hilum and opposite the area where the radicle emerges. Dislodging the plug (i.e. opening the water gap) is equivalent to breaking dormancy[28]. For Lamiaceae members, water gaps are formed as circular or narrow openings occluded by lid -like structures resulting from palisade cells equivalent to breaking dormancy[28]. For Lamiaceae members, water gaps are formed as circular or narrow openings occluded by lid-like structures resulting from palisade cells.

Seed coat anatomy and water gap regions were characterized using Scanning electron microscopy (SEM). Over the last two decades, the use of scanning electron microscopes has greatly enhanced our understanding of seed microstructures.

The objectives of this study involving *Ficus Benjamina* L., *Thespesia populnea* L., *Phyllanthus emblica* L., *Tectona grandis* L.f. seeds were threefold: (i) To corroborate reports of physical dormancy in Fabaceae seeds. (ii) To ascertain the mechanisms responsible for breaking dormancy and (iii) to pinpoint the location of the water gap during the process of dormancy break.

Material and Methods

Seed collection: The mature Fabaceae seeds of *Ficus Benjamina* L., *Thespesia populnea* L., *Phyllanthus emblica* L., *Tectona grandis* L.f. were collected from September to December 2020 from the Western Ghats, Sadivayal area in Coimbatore district (11.0168° N, 76.9558° E). The collection site has a mean annual temperature of 26°C, ranging from 32°C in September to 19°C in December. Several hundred seeds were collected from 10 randomly chosen trees located within a 20-km radius by gently shaking the mature trees.

Seeds were transported to the lab via road on the same day, visually inspected and any unhealthy seeds and debris, were separated. The seed surface was sterilized with 5% sodium hypochlorite solution for 10 minutes and thoroughly washed with sterile distilled water 4 to 5 times. The seeds were then bench dried for a day and subsequently stored in air-tight glass bottles under room temperature and humidity until used in the experiment.

Moisture content: Seed moisture content was measured using high constant temperature oven dry method following ISTA rules. The moisture content of fresh seeds was determined by using three replicates of 25 seeds: first by weighing them on a balance for initial weight and again weighing them after oven drying at 50 °C for 20 hours for dry weight. The final moisture content was expressed as percentage of fresh mass[14].

Dormancy assessment: Identification of seed dormancy was subjected to partially scarification followed by placing in hot air oven at 50°C for 15 minutes. Three replicates of 25 seeds were dipped in water, heated to 50 °C for 45 sec and allowed to cool to room temperature and then seeds were then placed on 4 inch Petri dishes lined by moist filter paper in 3’ inch Petri dishes and monitored for imbibition under ambient room conditions.

Imbibition: Water imbibition was determined for both hot water-treated seeds and control seeds. Take three replicates of 25 seeds each kept on Petri dishes and lined by filter paper maintained in ambient conditions. The seeds were initially weighed at intervals of 2 h as they imbibed water until 24 h and seeds were removed from the water, blotted dry on the surface, weighed, then returned to the water soak treatments until germination started.

The amount of absorbed water at each time was calculated using the equation:

$$\%Wi = ((Wi - Wf) / Wf) \times 100$$

where Wi is fresh weight after water absorption and Wf is initial fresh weight prior to imbibition[11,20].

Identification of water gap: To identify the water gap, after the dormancy break mechanism seeds were considered to be non-dormant seeds. The non-dormant and untreated seeds were scanned in a Scanning electron microscope and micrographs compared to identify changes in dormant seeds[14,15].

Statistical analysis: Data were analyzed by using SPSS software (IBM, version 21) to carry out one-way ANOVA ($P < 0.05$) for imbibition values. Values were expressed as means± SD.

Results and Discussion

Moisture content: Seeds were collected from four different species, each exhibiting varying moisture content. Among them, *Ficus benjamina* displayed the highest moisture content at 6.02%. This finding is consistent with glycine max seeds, which demonstrated the highest moisture at 7.08% in a study conducted by Ali et al[1,2].

Following this trend, *Phyllanthus emblica* had a moisture content of 5.31%, *Thespesia populnea* recorded 4.87% and *Abutilon pakistanicum* maintained a moisture content of 5.17%. The correlation between these moisture content levels in the mentioned species aligns with the moisture content findings in seeds of *Tectona grandis* which exhibited the least moisture content at 4.34%.

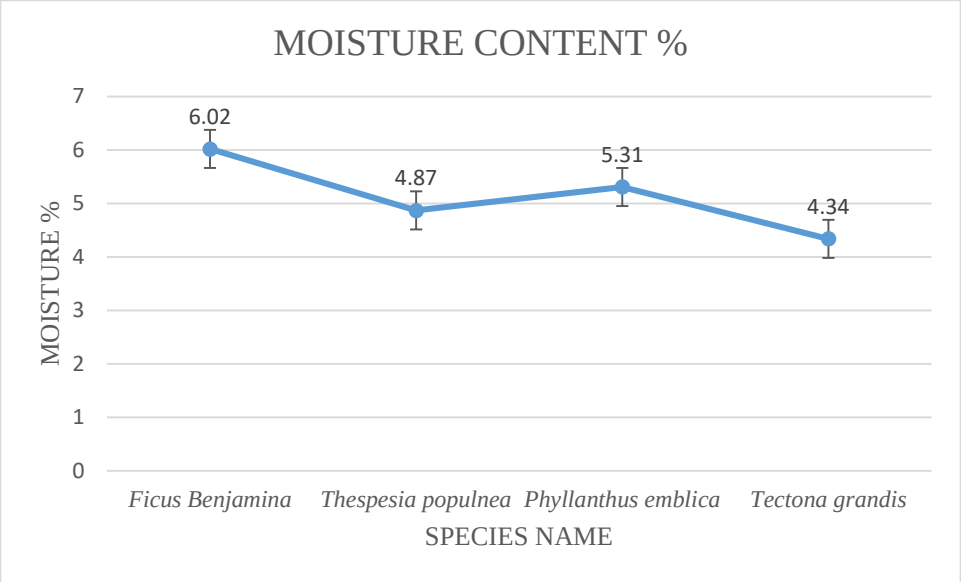

Fig. 1: Percentage of Moisture content of Four seeds

This result is positively linked to *Acacia pulchella* which showed a moisture content of 4.46%. This indicates that reducing moisture content and temperature can extend the storage life of seeds, as demonstrated in a study by Tangney et al[27]. The findings suggest the potential for enhanced seed preservation through moisture and temperature control strategies.

Dormancy assessments: The seeds underwent treatments involving partial scarification, hot air oven and hot-water treatment, all of which demonstrated a notable impact on breaking dormancy across the four seed types. In comparison to the control seeds, the treated seeds exhibited a significant increase in water absorption, exceeding 67%. This observation closely aligns with the specific physiological and morphological dormancy types prevalent in these seeds.

This outcome mirrors findings from earlier studies where treated seeds displayed higher water absorption compared to untreated seeds. This pattern is consistent and analogous to observations made in mimetic seeds, as detailed in studies by Jaganathan et al[14,15] in 2016 and 2018. These results collectively emphasize the effectiveness of the applied treatments in promoting dormancy break and water absorption, with parallels seen in seeds displaying mimetic behavior.

Imbibition: The seeds utilized in this study exhibited varying initial fresh weights (Wf). Among them, *Ficus benjamina* had the highest initial fresh weight of 56.84± 0.027g, followed by *Phyllanthus emblica* with an initial fresh weight of 41.23± 1.020g. *Tectona grandis* displayed an initial weight of 40.06 ± 1.64 while the lowest initial fresh weight was observed in *Thespesia populnea* measuring 34.22± 0.523g. Notably, previous research has shown a similar imbibition pattern in scarified seeds of *Serianthes kanehirae* and *Serianthes nelsonii*. These seeds displayed an imbibition rate ranging from 55% to 60% at the end of a 24 hour imbibition period, as reported in studies by Marler[20] in 2019 and Burrows et al[4]. This parallel imbibition behavior further highlights the consistent characteristics observed across different species and reinforces the findings of this study.

The fresh weight after water absorption (%Wi) indicated that all seeds underwent complete water uptake within the time frame of 15 to 24 hours. During this period, the rates of water uptake ranged from 35% to 65%. These results align positively with the observed water uptake pattern in seeds of Bolusanthus speciosus, *Combretum erythrophyllum*, *Erythrina caffra*. Similar patterns of water uptake were reported in the seeds of these species in studies conducted by Fatokun et al[11]. This consistency in water uptake behavior across different species reinforces the findings of this study and suggests a common trend in seed imbibition.

The control seeds maintained their initial fresh weight, with the highest seed mass observed in *Ficus benjamina* at 55.12± 0.050g. Conversely, the lowest fresh weight was recorded in *Thespesia populnea* measuring 34.22 ± 0.041g. This observation closely resembles the untreated seeds of *Adenanthera pavonina*, which did not absorb more in the hot water treatment compared to mechanically scarified seeds, as detailed in a study by Jaganathan et al. This connection between the two studies underscores the consistent behavior of untreated seeds in terms of water absorption, linking the findings of this study with prior research.

The water content and the time of imbibition showed a clear interrelation across all the species. The process of seed water intake exhibited a triphasic nature, characterized by three distinct phases - phase I, II and III as defined by Bewley and Black[3] in 1994.

By determining the seed imbibition curves, the appropriate

times for hor water treatment were established. Specifically, for *Ficus benjamina* seeds, the optimal imbibition time was 17 hrs, followed by *Thespesia populnea* and *Phyllanthus emblica*, which both exhibited an imbibition time of 19 hours. On the other hand, *Tectona grandis* seeds required 23 hours for proper imbibition.

This triphsic pattern of seed water uptake observed in our study aligns with previous findings by various authors. For instance, *Pisum sativum* and *Cucurbita pepo* seeds also displayed a similar imbibition duration ranging from 18 to 24 hrs, as documented in studies by Fatokun et al[11] and Ali et al[1]. The consistency in the triphasic pattern across different species further strengthens the validity of the observed imbibition behavior in this study.

Table 1
Imbibition Values as Means± SD

Species	Initial Fresh Weight(g) (Wf)	Initial Fresh Weight (Wi) (g) (At end of 24 hours)	%Wi = ((Wi – Wf) / Wf) × 100 (g)	Non-treated Seeds (g) INITIAL Weight (At end of 24 hours)
Ficus Benjamina	56.84± 0.027*	80.23± 0.036	41.15± 0.027*	55.12± 0.050
Thespesia populnea	34.22± 0.523*	55.85 ± 0.467	63.20. ± 0.564*	34.22 ± 0.041
Phyllanthus emblica	41.23± 1.020*	60.58± 0.276	46.08± 0.431*	40.4 ± 0450
Tectona grandis	40.06 ± 1.64*	56.74 ± 2.085	41.85± 2.046*	40.05± 0.132

Asterisk (*) indicates significantly greater than the control value (P <0.05)

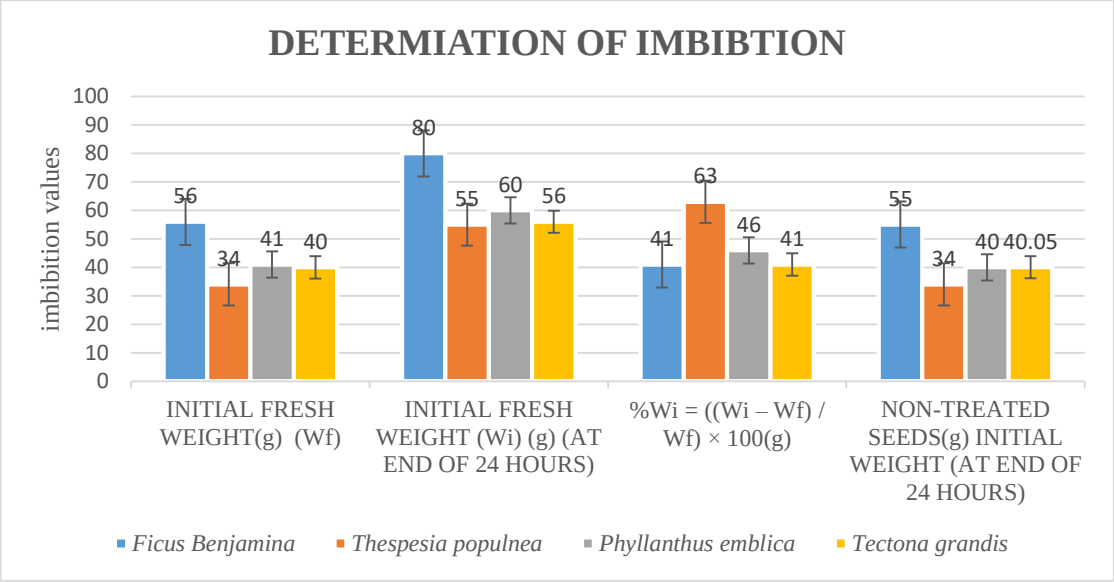

Fig. 2: Imbibition rate of four seeds

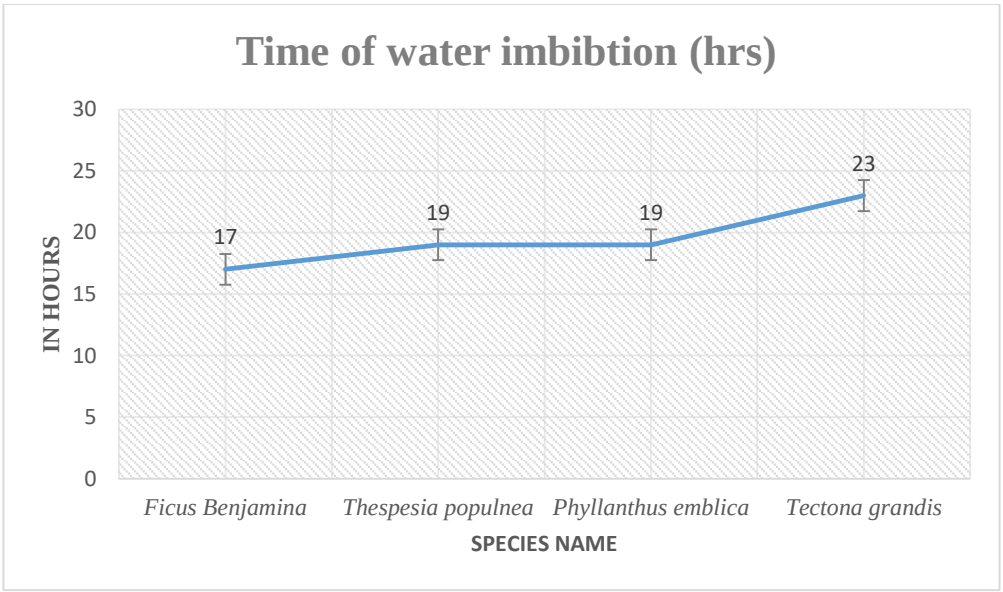

Fig. 3: Time of imbibition (h)

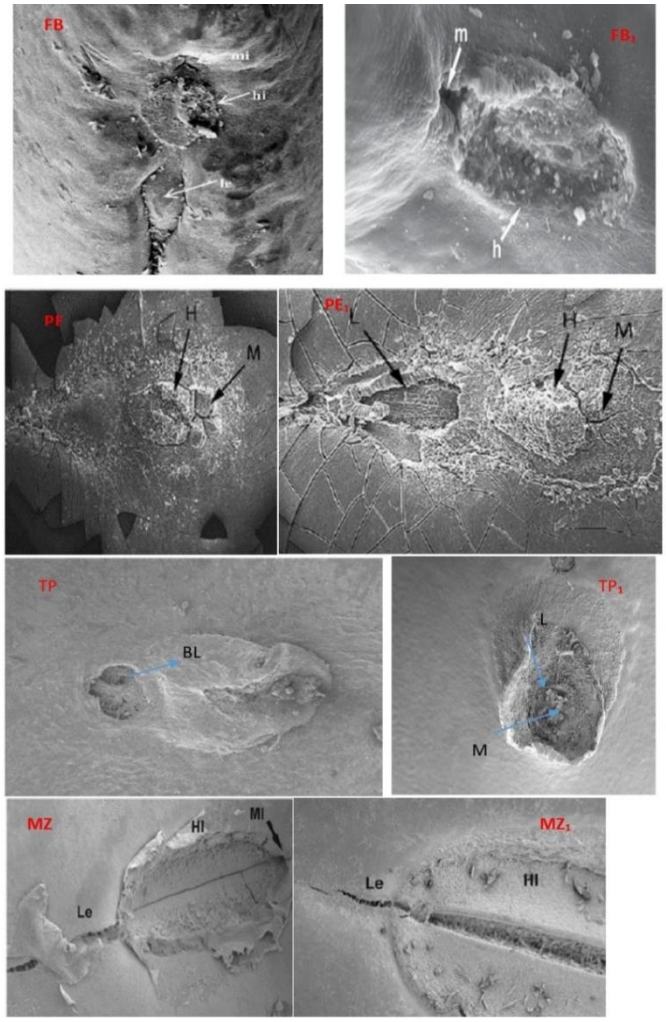

Fig. 4: Electron micrographs of FB (dormant seed) and FB1 (non-dormant seed) *Ficus Benjamina* seeds; PE (dormant seed) and PE1 (non-dormant seed) *Phyllanthus emblica* seeds; TP (dormant seed) and TP1 (non-dormant seed) *Thespesia populnea* seeds; MZ (dormant seed) and MZ1 (non-dormant seed) *Tectona grandis* seeds. Abbreviations: B, bulge; ED, endodermal cells; HF, hilar fissure; H, hilum; HP, hilar pad; HR, hilar ring; MP, micropyle; L, lens.

Identification of water gap: SEM analysis was conducted to identify the water gap and notable differences were observed between control seeds and seeds subjected to hot water treatment. In control seeds, no discernible characteristics of a lens region were evident. However, in seeds treated with hot water, the lens region was visibly opened due to dislodgement. This phenomenon was particularly observed in seeds of *Ficus benjamina* and *Thespesia populnea*, where the lens was situated on the opposite side of the micropyle and palisade layer. In the case of *Phyllanthus emblica* and *Tectona grandis*, the lens region exhibited a circular opening pattern while the hilum remained unchanged and no visible cracks were observed after hot-water treatment.

These results closely mirror the findings in *Adenanthera pavaonia*, where treated seeds displayed similar characteristics with an opened lens area, while control seeds did no exhibit such changes. This comparison aligns with observations made by Lersten[18] in 1992. The SEM analysis thus confirms the presence of distinct water gap features and their response to hot water treatment in the seeds under investigation, shedding light on the mechanisms involved in breaking dormancy.

Conclusion

In conclusion, the current study successfully established effective techniques for breaking dormancy in four different seed species. Scarification and hot water treatments were identified as viable methods for achieving dormancy break. These treatments led to the discernible opening of the lens region, primarily attributed to the dislodgement of the palisade layer, which serves as the initial point of water entry into the seeds. Furthermore, the extended storage life of the four seed species was demonstrated through reduced moisture content. These tests collectively contribute to enhancing the overall germination and storage potential of the seeds.

Overall, the findings of this study not only provide practical

strategies for breaking dormancy but also offer insights into the physiological processes underlying seed germination and preservation. This knowledge has the potential to contribute to the maintenance of high germination rates and optimal storage conditions for these seeds, ultimate;y benefiting agricultural and conservation efforts.

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(Received 02nd October 2023, accepted 06th December 2023)