

Investigation of Dormancy Breaking and Water Uptake Mechanisms in *Majidea zangueberica* Kirk ex Oliv., *Markhamia lutea* (Benth.) K. Schum., *Tabebuia aurea* (Silva Manso) Benth. and Hook. f. ex S. Moore, *Santalum album* L., and *Morinda citrifolia* L. seeds.

Preethi Jenifer Praticia. S and Kanchana. M*

Department of Botany, PSGR Krishnammal College for Women, Coimbatore, Tamil Nadu, INDIA.

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ABSTRACT

Aim: The primary aim of this research is to analyze the conditions that prompt dormancy release and identify the primary water gap location during the dormancy-breaking process. By subjecting the seeds to various dormancy-breaking techniques, we observed that water ingress occurred exclusively through the lens due to the dislodgement of two sclereid layers. **Background:** Water uptake is a vital process for seed germination. However, in these seeds, a water-impermeable seed coat, while beneficial for adapting to the environment, hinders germination without appropriate treatment. Nevertheless, the factors triggering dormancy release, along with varying interpretations of the specialized seed coat structure known as the “water gap”, remain unresolved for seeds such as *Majidea zangueberica* Kirk ex Oliv., *Markhamia lutea* (Benth) K. Schum., *Tabebuia aurea* (Silva Manso) Benth. and Hook.f.ex S. Moore, *Santalum album* L., and *Morinda citrifolia* L. **Materials and Methods:** Seed moisture content was measured using a high constant temperature oven dry method followed by dormancy assessment and identification of water gap by SEM analysis. **Results:** The storage life of *Tabebuia aurea* seeds was extended, substantiated by a notably low moisture content of 4.22%, here by SEM analysis the lens regions adopted circular or lid-like shapes, maintaining structural integrity in the hilum and micropyle regions whereas in *Majidea zangueberica*, where a blister-like aspect resembling. **Conclusion:** This research has provided details on the period in which seeds with impermeable seed coverings and low moisture content can be stored. These can be seen to time their germination to best coincide with the growing season.

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

Correspondence:

Kanchana. M

Associate Professor,
Department of Botany,
PSGR Krishnammal
College for Women,
Coimbatore, Tamil Nadu,
INDIA.

Email: kanchana09
psgrkcw@gmail.com

INTRODUCTION

In otherwise optimal conditions (such as high moisture, light, and temperature), dormancy inhibits seeds from germinating as quickly as they would if they were

not dormant. Morphological (MD), Physical (PY), Morphophysiological (MPD), Physiological (PD), and combinational dormancy (PY+PD) were the five categories of dormancy.^[1,2]

The typical techniques for promoting the germination of dormant seeds include the use of several growth regulators, including Gibberellins, Cytokinin, and Ethylene, along with prechilling, scarification, nitric acid treatments, and pre-chilling.^[2] Seeds of *Bituminaria basaltica*, *Ormosia arborea*, and *Luffa cylindrica* can be physically awakened through mechanical and chemical scarification treatments.^[3] In various species, such as *Rheum webbianum*,

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Carum carvi, *Saussurea lappa*, and *Bunium persicum* the exogenous administration of gibberellic acid eliminates seed dormancy.^[4] Gibberellic acid and cold stratification are two more efficient ways to awaken dormant seeds of *Brassica tournefortii* and *Ducrosia anethifolia*.^[5]

During the maturation drying process, the seed coat becomes impermeable to water.^[6,7] This impermeability in the seed coat is caused by palisade layers. When an opening is created in the palisade layers through a specific anatomical structure called a “water gap,” water can enter the seed and cause it to become permeable to water. Of the 16 families reported to have PY, 12 have described a water gap. Additionally, seeds belonging to these 12 families have various types of water gaps that vary in origin, morphology, and anatomy.^[8]

Water gaps can be encountered in the capillary hilar or micropylar areas of the pericarp as well as the chalaza, micropylar, or hilar sections of the seed.^[8] Multiple morpho-anatomical structures are frequently linked to the water gap, resulting in specialized regions known as water-gap complexes.^[9] As a result, one or more morpho-anatomical structures that are closely related to the gap opening (water gap) make up a water gap complex. Water-gap complexes absorb signaling from the environment and identify conditions that are favourable for plant establishment and seed germination.^[10] Three categories apply to the water-gap complexes: Type II water gaps are circular or narrow and are occluded by lid-like structures that result from palisade cells; Type III water gaps are either narrow or circular and are occluded by plug-like structures that result from sclerenchyma cells. Type I water gaps have narrow linear openings that are occluded by modified elongated palisade cells.^[11,12]

The objectives of this study involve *Majidea zangueberica* Kirk ex Oliv., *Markhamia lutea* (Benth.) K. Schum., *Tabebuia aurea* (Silva Manso) Benth. and Hook. f. ex S. Moore, *Santalum album* L., and *Morinda citrifolia* L. seeds were threefold:

- (i) To corroborate reports of physical dormancy in these seeds
- (ii) To ascertain the mechanisms responsible for breaking dormancy.
- (iii) To figure out where the water gap is during the dormancy break process.

MATERIALS AND METHODS

Seed collection

The mature seeds of *Majidea zangueberica*, *Markhamia lutea*, *Tabebuia aurea*, *Santalum album*, and *Morinda citrifolia* were collected from September to December 2020

from the Western Ghats, Sadivayal area in Coimbatore district (11.0168° N, 76.9558° E). The average annual temperature at the collecting location is 26°C, with monthly variations from 32°C in September to 19°C in December. A few hundred seeds were harvested by gently shaking ten randomly selected mature trees that were spread out across a 20-kilometre radius. The following day, the seeds were driven to the laboratory, where they were visually inspected, any diseased seeds were removed, and debris was also separated. Following sterilizing the seed surface for 10 min with a 5% sodium hypochlorite solution, the seed surface was carefully cleaned four to five times using sterile distilled water. 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

The high constant temperature oven dry method^[13] was used to measure the moisture content of the seeds. Three replicates of twenty-five seeds each were used to calculate the moisture content of fresh seeds. To determine their dry weight, weigh them once more after 20 hr of oven drying at 50°C. Weigh them first using a balance to determine their initial weight. A percentage of fresh mass was used to express the final moisture content.^[14]

Dormancy assessment

Identification of seed dormancy was subjected to partial scarification followed by placing a hot air oven at 50°C for 15 min. Three duplicates of twenty-five seeds each were submerged in water heated to 50°C for forty-five seconds and then allowed to cool to room temperature. The seeds were then arranged in four-inch Petri dishes lined with moist filter paper in three-inch Petri dishes, and the imbibition test was observed in ambient room conditions using this technique for breaking dormancy for *Majidea zangueberica*, *Markhamia lutea*, *Tabebuia aurea*, *Morinda citrifolia*. To prepare the seeds for the *Santalum album*, they were first partially scarified, then placed between damp towels at 4°C for 21 days, heated in a hot air oven for 3 min at 50°C, and then dipped in water heated to that same temperature for 45 seconds before being allowed to cool to room temperature. Finally, the seeds were placed on 4½ Petri dishes lined with moist filter paper in 3 inch Petri dishes, and their imbibition was observed under ambient room conditions.^[15]

Imbibition

Water imbibition was determined for both hot water-treated seeds and control seeds. Take three replicates of 25 seeds each which are kept on petri dishes and lined

by filter paper maintained in ambient conditions. The seeds were first weighed every 2 hr while they soaked in water for 24 hr. After that, they were taken out of the water, blotted dry on the surface, weighed again, and put back into the water-soaked treatments until germination began.

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

$$\%W_i = ((W_i - W_f) / W_f) \times 100$$

Where W_i is the fresh weight after water absorption and W_f is the initial fresh weight earlier to imbibition.^[16]

Identification of water gap

Following the dormancy break mechanism, seeds were deemed to be non-dormant and used to determine the water gap. Using a scanning electron microscope, the non-dormant and untreated seeds were scanned, and micrographs were compared to determine alterations in dormant seeds.^[17,18]

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 mean \pm SE.

RESULTS

Moisture content

In the context of the current study involving seeds of *Majidea zangueberica* Kirk ex Oliv., *Markhamia lutea* (Benth.) K. Schum., *Tabebuia aurea* (Silva Manso) Benth. and Hook. f. ex S. Moore, *Santalum album* L., and *Morinda*

citrifolia L., it is noteworthy that *Santalum album* exhibited the highest moisture content at 13.02%. The moisture content patterns persisted in other studied species as well. *Morinda citrifolia* demonstrated a moisture content of 7.54%, while *Majidea zangueberica* recorded 6.66% and *Markhamia lutea* maintained a moisture content of 6.59%. This consistent correlation of moisture content levels within the mentioned species is further supported by the moisture content findings in seeds of *Tabebuia aurea*, which exhibited the lowest moisture content at 4.22% (Figure 1).

Dormancy assessments

The impact of treatment on breaking dormancy in the seeds was striking, leading to a significant uptake of more than 70% water compared to control seeds. This effect resonates strongly with the underlying dormancy type observed in different species. Specifically, the physiological dormancy present in *Majidea zangueberica*, *Markhamia lutea*, *Tabebuia aurea*, and *Morinda citrifolia* seeds, and the morphophysiological dormancy in *Santalum album*, play a pivotal role in influencing water absorption behaviour.

Imbibition

The assessment of water content as a percentage unveils compelling interspecies disparities concerning imbibition times and seed water contents. This observation prompts a closer examination of the initial fresh weights (W_f) of the seeds under study. Among these seeds, *Morinda citrifolia* exhibited an initial fresh weight of 54.84 ± 0.017 g, closely followed by *Santalum album* at 40.23 ± 0.020 g. *Majidea zangueberica* displayed an

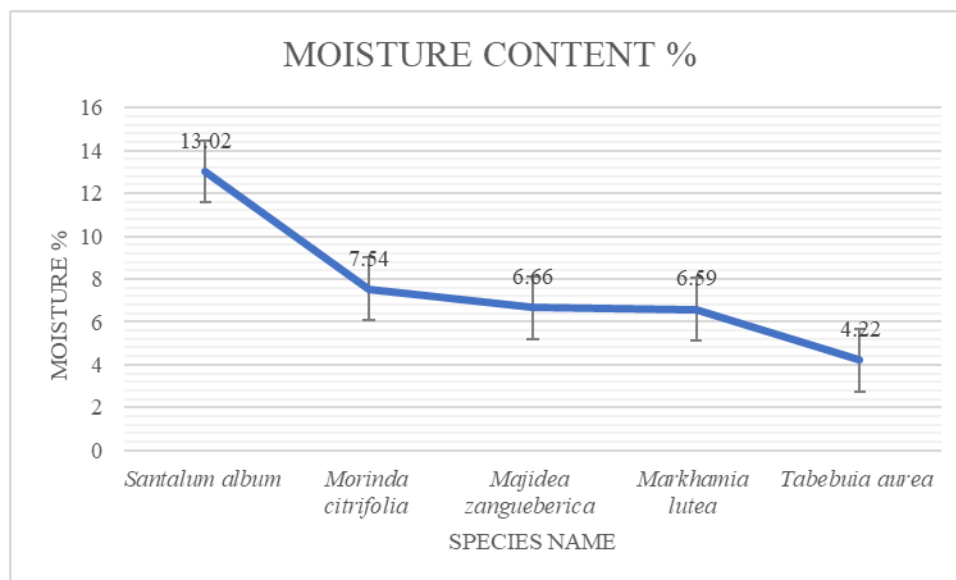


Figure 1: Percentage of Moisture content of seed.

Table 1: Imbibition values as Means±SE.

Species	Initial Fresh Weight (G) (Wf)	Initial Fresh Weight (Wi) (G) (At end of 24 hr)	%Wi= $((Wi-Wf)/Wf) \times 100(G)$	Non-Treated Seeds(G) Initial Weight (At end of 24 hr)
<i>Morinda citrifolia</i>	54.84±0.017*	80.23±0.036	41.15±0.027*	55.12±0.050
<i>Tabebuia aurea</i>	34.22±0.523*	55.85±0.467	63.20±0.564*	34.22±0.041
<i>Santalum album</i>	40.23±0.020*	60.58±0.276	46.08±0.431*	40.4±0.450
<i>Markhamia lutea</i>	37.41±1.541*	49.86±0.320	33.27±1.024*	37.11±0.471
<i>Majideia zangueberica</i>	39.06±1.64*	56.74±2.085	41.85±2.046*	40.05±0.132

Asterisk (*) indicates significantly greater than the control value ($p < 0.05$).

initial fresh weight of 39.06±1.64 g, whilst *Markhamia lutea* weight was measured at 37.41±1.541 g. Notably, the lowest initial fresh weight was ascertained for *Tabebuia aurea*, registering at 34.22±0.523 g (Table 1).

The examination of fresh weight after water absorption (%Wi) offers a glimpse into the temporal dynamics of seed imbibition. In our study, a consistent trend emerged, as water uptake was predominantly observed within the 15 to 24 hr window, encompassing approximately 35% to 65% of the total water absorption (Table 1).

The consistency in imbibition behaviours across diverse tree species holds significant implications. It suggests the existence of shared physiological mechanisms that regulate the temporal dynamics of water uptake during germination. In addition, when considering the initial fresh weights of control seeds, our findings reflect a continuum of mass variations. *Morinda citrifolia* demonstrated the highest seed mass at 55.12±0.050 g, while *Tabebuia aurea* displayed the lowest fresh weight at 34.22±0.041 g (Figure 2, Table 1).

The utilization of seed imbibition curves for the determination of optimal timing for hot water treatment marked a significant application of our research. Notably, *Morinda citrifolia* seeds exhibited a unique imbibition pattern requiring 17 hrs for complete water uptake, while *Tabebuia aurea* and *Markhamia lutea* seeds mirrored each other, necessitating 19 hrs. In contrast, the seeds of *Santalum album* and *Majideia zangueberica* followed an extended path of 23 hr for imbibition. These tailored timeframes for water uptake provide crucial insights for effective dormancy-breaking strategies (Figure 3).

Identification of water gap

The utilization of SEM analysis was instrumental in unravelling the intricacies of water gap dynamics, shedding light on the structural transformations occurring within the seeds' lens regions. Control seeds failed to manifest any distinct attributes in the lens area, whereas seeds subjected to hot water treatment

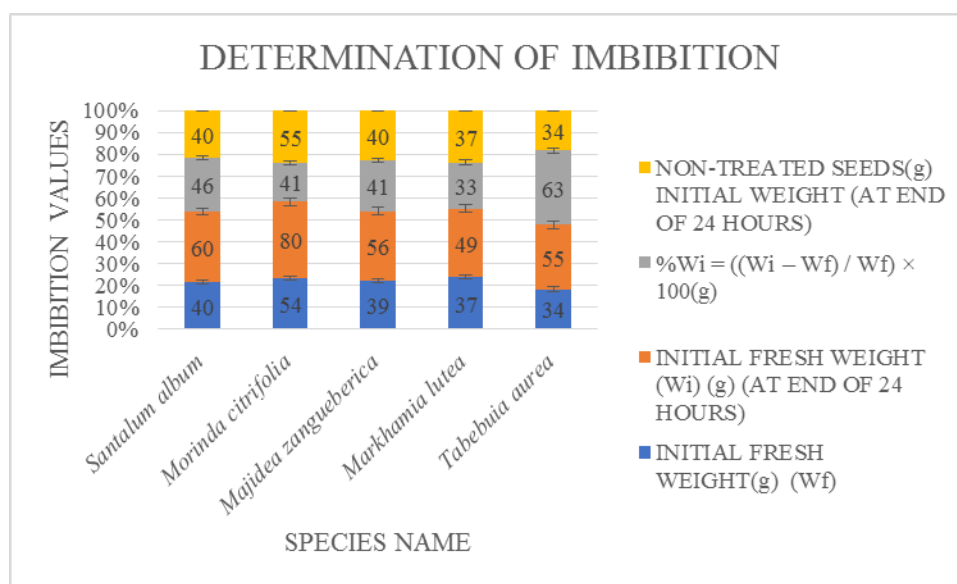


Figure 2: Imbibition rate of seeds.

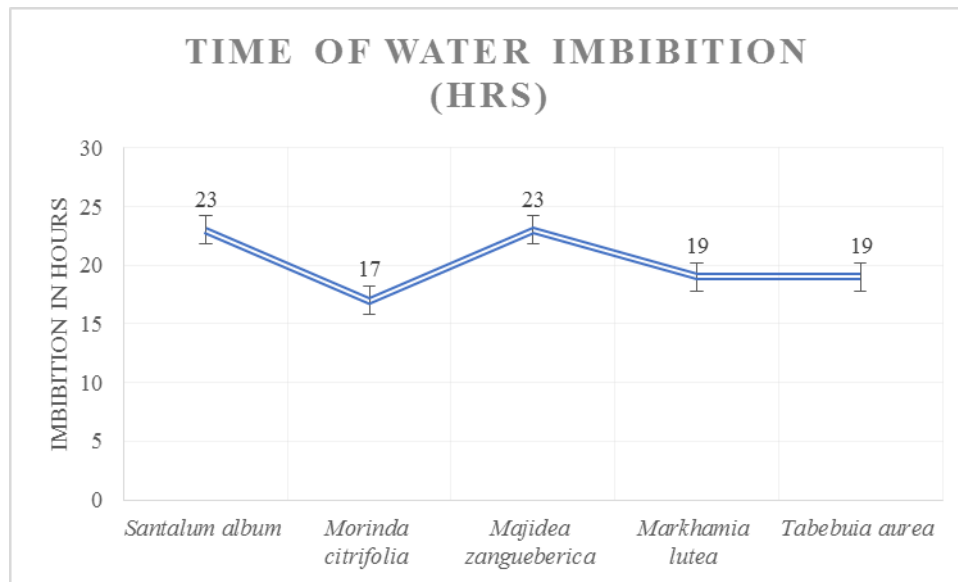


Figure 3: Time of imbibition (h).

exhibited discernible alterations. Notably, the lens region in seeds of *Morinda citrifolia* and *Santalum album* displayed a pivotal transformation, as the two sclereid layers dislodged, allowing the lens region to open. Intriguingly, this phenomenon was observed without any observable differentiation in the hilum region compared to the other seeds.

Analogous observations in *Markhamia lutea* and *Tabebuia aurea*, further enrich the narrative. Here, the lens regions adopted circular or lid-like shapes, hilar slit, maintaining structural integrity in the hilum and micropyle regions. In these species, the lens becomes an important anatomical feature because it serves as the main site of entry for water and germination stimulation.

A distinctive finding emerged in *Majideia zangueberica*, where a blister-like aspect (plug-like structure) resembling a lens was discernible and subsequently dislodged. The positioning of this blister, adjacent to the hilum on the opposite side of radicle emergence, further highlights the intricate relationship between anatomical structure and seed germination (Figure 4).

DISCUSSION

Interestingly, this observation aligns with a separate study conducted by^[19] which found that *Glycine max* seeds possessed the highest moisture content at 7.08%, similar way studies on *Nelumbo nucifera*, *Lupinus digitatus*, and *Trifolium ambiguum* further show that the impermeability of seeds at 7.14% moisture content can be reversed when the seeds are exposed to high ambient

relative humidity. This exposure ultimately increases the moisture content of the seeds. This supports the previous findings that impermeability can be reversed after seeds become impermeable as reported.^[20] The commonality in moisture content trends across different seed species underscores the significance of moisture as a crucial factor governing seed characteristics. Notably, *Acacia pulchella*, displaying a moisture content of 4.46%, reaffirms the link between reduced moisture content and potential extended seed storage life. Similarly, showed that *Achnatherum inebrians* seeds have increasing seed moisture content (5.56%) it clearly demonstrating the importance of seed moisture to the viability of seed.^[21] At very low moisture contents (i.e., for seeds preconditioned at 15% RH), physiological reactions in seeds are constrained due to the extreme viscosity of the water.^[22]

It is intriguing to note that the implications of moisture content on seed storage life have been explored in previous research.^[20] demonstrated that by strategically reducing moisture content and controlling temperature, seeds can undergo an extended storage life. This reinforces the notion that moisture content regulation, in conjunction with temperature management, plays a pivotal role in enhancing seed preservation potential.

In essence, the present findings underscore the significance of moisture content in seed physiology and storage longevity. The parallels between these results and those from other studies support the notion that

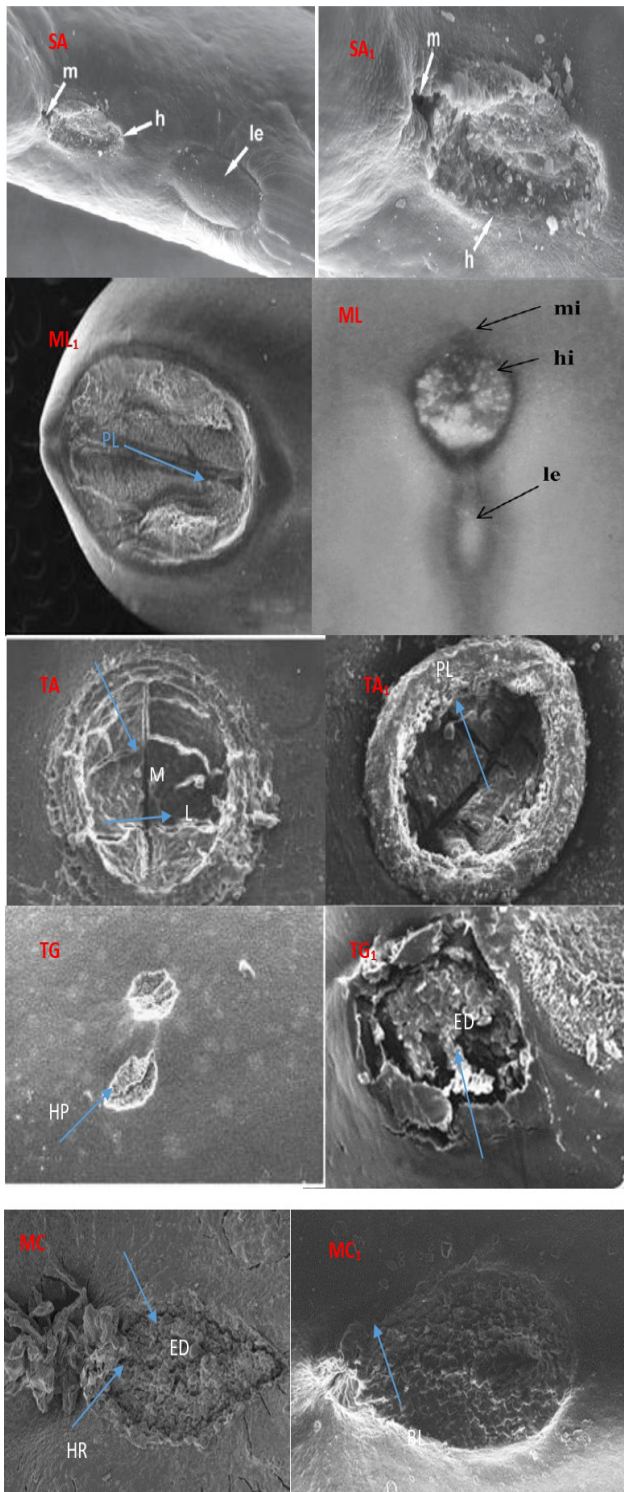


Figure 4: SEM images of SA (dormant seed) and SA1 (non-dormant seed) *Santalum album* seeds; ML (dormant seed) and ML1 (non-dormant seed) *Markhamia lutea* seeds; TA (dormant seed) and TA1 (non-dormant seed) *Majidea zangueberica* seeds; MC (dormant seed) and MC1 (non-dormant seed) *Morinda citrifolia* seeds and TG (dormant seed) and TG1 (non-dormant seed) *Tabebuia aurea* seeds. Abbreviations: B, bulge; ED, endodermal cells; HF, hilar fissure; H, hilum; HP, hilar pad; HR, hilar ring; MP, micropyle; L, lens.

targeted manipulation of moisture and temperature conditions holds promise for augmenting seed preservation strategies.

Dormancy assessments

Importantly, this aligns with earlier research where treated seeds consistently exhibited higher water uptake levels than untreated counterparts. Notably, similar trends were also observed in mimetic seeds, as documented by.^[17,18]

In a comparable manner,^[19] seeds of the wild chickpea parent had a thicker macrosclereid palisade layer, which probably contributes to the water impermeability of seed coats. However, the structural differences of seed coats are not the only major factor. Rather, the chemical composition of the test seems to be the most important factor.

The effect of the maternal environment on seed dormancy has been shown^[25] in *Arabidopsis* and other plants with physiological dormancy types, but it is likely expected also in physical dormancy, in which it was found that the release of physical dormancy of *Medicago truncatula* from arid regions occurred to a greater extent under higher (35/15°C) rather than lower (25/15°C) temperature alternations.^[26] In addition, the position of seed development on the parental plant predominantly influences the physiological dormancy in Brassicaceae^[27] and can be expected to affect dormancy in many other plant families, including legumes.

This emerging consistency across diverse species and studies highlights the robustness of the observed phenomenon. Moreover, it underscores the importance of dormancy type, as well as the efficacy of treatment, in modulating water uptake dynamics. These findings collectively underscore the potential applicability of dormancy-breaking techniques, informed by these insights, to a broader range of plant species, contributing to enhanced germination and seedling establishment.

Imbibition

This observation resonates with the behavior of untreated seeds of *Adenanthera pavonina*, which exhibit reduced water absorption during hot water treatment compared to mechanically scarified seeds.^[14]

The correlation between water content and the duration of imbibition emerged as a salient feature across all studied species. Notably, the seed water intake manifested a triphasic path, delineated into distinctive phases I, II, and III, as elucidated by.^[21] This nuanced insight into the imbibition process underscores the complexity and multi-stage nature of water absorption during germination.

The intricate tapestry of initial weights is a key determinant shaping subsequent imbibition kinetics. Interestingly, this phenomenon resonates intriguingly with findings from earlier research. Investigations into the imbibition behaviors of scarified seeds, particularly those of *Serianthes kanehirae* and *Serianthes nelsonii*, stand as pertinent examples. These studies revealed a consistent imbibition pattern, marked by water uptake ranging from 55% to 60% within a 24 hr period.^[15,22]

The convergence of our observations with those of^[15,22,30] underscores the cross-species applicability of certain imbibition kinetics. The shared pattern of reaching a specific water uptake percentage within a designated timeframe transcends species boundaries. By weaving these findings together, we recognize the broader principles that govern water absorption in tree seeds.

The harmonization of our research with prior studies accentuates the significance of understanding imbibition within a wider ecological context. The patterns that emerge, spanning various species and studies, signify an overarching theme within seed hydration dynamics, laying the foundation for more comprehensive insights into seed germination processes.

Surprisingly, this imbibition characteristic matches findings from other tree species in an intriguing way. Comparing the results of studies conducted on the seeds of *Combretum erythrophyllum*, *Erythrina caffra*, and *Bolusanthus speciosus*,^[16,23] reveals a striking congruence. These species exhibited analogous water uptake kinetics, with a prominent water absorption phase within the 15 to 24 hr timeframe, accompanied by a comparable range of water uptake percentages.

By intertwining our outcomes with those of^[16,17,23] we delineate a comprehensive understanding of seed imbibition dynamics. The shared patterns in water uptake underscore universal aspects of germination processes, while also emphasizing the importance of considering initial seed attributes when deciphering imbibition behavior.

In essence, our study's findings emphasize a recurring pattern in the fresh weight after water absorption (%Wi), mirrored across distinct tree species. The integration of research outcomes with those of analogous studies advances our knowledge of imbibition kinetics and accentuates the significance of investigating the shared physiological drivers underlying these phenomena.

The observed triphasic pattern of seed water uptake aligns intriguingly with precedent studies. The phenomenon of imbibition spanning phases I, II, and III has been noted previously and lends credence to the

robustness of our findings. Notably, analogous patterns were discovered in seeds of *Pisum sativum* and *Cucurbita pepo*, substantiated by research conducted by.^[16,24] These species showcased imbibition durations spanning the range of 18 to 24 hr, mirroring the triphasic nature of water absorption observed in our study.

Corte-Real *et al.*^[34] designate that the hard seed coat species presented a smooth and long phase I and shows an absence or a very short phase II, i.e., in *Euterpe oleracea* and *Spondias tuberosa* seeds the full imbibition seems to be completed after 72 hr. Though, the stabilization of water intake is complete after 24 hr in *Cucurbita maxima* and *Jatropha curcas*.

In some Leguminosae species, the seed coat is highly permeable promoting fast water imbibitions,^[31,32] a fact confirmed in *Arachis hypogaea*, where the seed imbibition is complete after the first 24 hr. In another way, *Prosopis juliflora* the presence of endocarp strongly attached seeds, leads to complete germination delay due to the impermeability of the seed coat.^[33]

The parallelism in imbibition patterns across diverse plant species underscores the conserved nature of seed hydration mechanisms. This uniformity reflects the fundamental physiological processes governing water uptake during germination, regardless of species boundaries.

In conclusion, our study advances our understanding of seed imbibition dynamics by revealing the interconnectedness between water content and imbibition duration. The triphasic pattern we observed not only enriches our comprehension of the imbibition process but also finds resonance in previous research. By integrating our findings with the insights of,^[16,21,24] we contribute to the broader discourse on seed germination kinetics and the regulatory mechanisms underlying this critical phase.

The water potential values in a quiescent seed are very variable, lying between -50 MPa and -400 MPa, producing a relatively high-water gradient between the seed and the imbibition water (~0 MPa). In conformity with Moncaleano-Escandon,^[32] the deterioration process begins with the loss of selective permeability of the cellular membranes and ends with the loss of the germination capacity. On other hand,^[31] this pattern is true in general species, which diverges from the profile of *Malpighia glabra* and *Prosopis juliflora*, which despite showing a slight tendency to stabilize, this water imbibition did not completely occur within the first 120 hr of imbibition. At the commencement of imbibition, the matric component of the seeds is the main component liable for the movement of water,

tending to increase as free water availability and the seed metabolism, within the osmotic component increases its participation in the process.^[30]

Water is circulated in crevices, cracks, and faults in the seed cover, and is absorbed by the seed tissues.^[29]

Water-uptake measurements, taken during this phase have shown these changes to be: (1) temperature-dependent; and (2) escorted by increases in respiration rate and in light sensitivity in some seed species.^[32]

These observations suggest that water uptake during imbibition is not a 'passive' process, as it is usually taken to be, but becomes an active one at an early stage of this phase, maybe promoted by aquaporins.^[33] This phase is noticeable by an asymptotic approach to a final water gain, or hydration level, which rest on on ambient soil–water potential, the conductivity of the soil to water, the seed–soil contact, and seed composition.^[31]

Identification of water gap

Interestingly, these findings closely mirror observations made in *Adenanthera pavonina*, where treated seeds exhibited akin characteristics, while control seeds displayed no lens area opening.^[24] This congruence reaffirms the significance of our results and underscores the generalizability of lens region behavior across species subjected to dormancy-breaking treatments.

These findings are consistent with research published by,^[24] which found that dormancy-breaking therapies elevated palisade layers in the lens region, which resulted in the creation of a water gap, which is a circular lid-like opening. Comparably,^[25] characterized the water-gap region of Convolvulaceae and called it the bulge gap adjacent to the micropyle, except for *Cuscuta*, in which the water gap is the hilar slit. However, the two bulges are located on the opposite ends of the hilar slit.

The water-gap region of *Koelreuteria paniculata* is comparable in numerous ways to the chalazal oculus of Bixaceae, Cistaceae, Malvaceae, Sarcocaulaceae, and Sphaerosepalaceae. In this seed, the water gap is occluded by a plug-like structure formed by water-impermeable sclerenchyma cells.^[26] In the family Surianaceae, only the genus *Stylobasium* has been shown to contain a water-impermeable endocarp.^[27] The water gap of *Stylobasium* is rather different from all the other water-gap regions. Unlike water-gap regions in other taxa, a suture (endocarp circumlinear suture) is formed all around the endocarp as PY is broken. Moreover, compared with other species the water-gap complex of *Stylobasium* is morpho-anatomically simple.

The integration of our SEM analysis outcomes with the insights of^[23] engenders a cohesive understanding of water gap dynamics across a spectrum of species.

The recurrence of specific structural transformations post-dormancy breaking underscores the conserved nature of these processes, and by extension, the adaptive mechanisms seeds employ to trigger germination in response to environmental cues.

In essence, our SEM analysis has illuminated the intricate interplay between anatomical alterations and water gap formation. By contextualizing our findings within the framework established by,^[24] we contribute to the elucidation of the underlying mechanisms governing water gap dynamics across species, enhancing our comprehension of seed germination initiation.

CONCLUSION

The results of the current study were utilized to develop a hot air oven technique for breaking dormancy in these seeds. Following the dormancy-breaking treatment, the identified lens region was characterized by the opening of the two sclereid layers through which water initially enters the seeds. Additionally, a decrease in moisture content demonstrated an extension in the storage life of these seeds. Through these tests, this technique could aid in maintaining high germination rates and storage capacity in the seeds.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

ABBREVIATIONS

PY: Physical dormancy; **SEM:** Scanning electron microscope; **%Wi:** Fresh weight percentage

SUMMARY

Under otherwise ideal circumstances (such as high moisture, light, and temperature), which would promote germination if seeds were not dormant, germination is postponed by dormancy. There were five distinct forms of dormancy. During the maturation drying process, the seed coat becomes impermeable to water. This impermeability in the seed coat is caused

by palisade layers. When an opening is created in the palisade layers through a specific anatomical structure called a “water gap,” water can enter the seed and cause it to become permeable to water. During this study, the moisture content patterns of *Santalum album* exhibited the highest moisture content at 13.02% and *Markhamia lutea* maintained a moisture content of 6.59%. The impact of treatment on breaking dormancy in the seeds was striking, leading to a significant uptake of more than 70% water compared to control seeds after dormancy breaking the lens region in seeds of *Morinda citrifolia* and *Santalum album* displayed a pivotal transformation, as the two sclereid layers dislodged, allowing the lens region to open followed by observations in *Markhamia lutea* and *Tabebuia aurea*, the lens regions adopted circular or lid-like shapes, maintain structural integrity in the hilum and micropyle regions and *Majidea zangneberica*, where a blister-like aspect resembling a lens was discernible and subsequently dislodged these characters were visible during SEM analysis.

REFERENCES

- Chen D, Zhang R, Baskin CC, Hu X. Water permeability/impermeability in seeds of 15 species of *Caragana* (Fabaceae). PeerJ. 2019;7:e6870. doi: 10.7717/peerj.6870, PMID 31119080.
- Chen JZ, Huang XL, Xiao XF, Liu JM, Liao XF, Sun QW, et al. Seed Dormancy Release and Germination Requirements of *Cinnamomum migao*, an Endangered and Rare Woody Plant in Southwest China. Front Plant Sci. 2022;13:770940-. doi: 10.3389/fpls.2022.770940, PMID 35154219.
- Carruggio F, Onofri A, Impelluso C, Giusso del Galdo G, Scopece G, Cristaudo A. Seed dormancy breaking and germination in *Bituminaria basaltica* and *B. bituminosa* (Fabaceae). Plants (Basel). 2020;9(9):1110. doi: 10.3390/plants9091110, PMID 32867404.
- Molizane DM, Carmello-Guerreiro SM, Barbedo CJ. Induction of seed coat water impermeability during maturation of *Erythrina speciosa* seeds. J Seed Sci. 2020;42. doi: 10.1590/2317-1545v42228614.
- Chaodumrikul S, Kaeworn P, Chulaka P, Chanprasert W. Breaking seed dormancy in smooth loofah (*Luffa cylindrica* (L.) M. Roem.) using scarification and dry heat treatment. Agric Nat Resour. 2016;50(2):85-8. doi: 10.1016/j.anres.2015.09.003.
- Turner SR, Cook A, Baskin JM, Baskin CC, Tuckett RE, Steadman KJ, et al. Identification and characterization of the water gap in the physically dormant seeds of *Dodonaea petiolaris*: a first report for Sapindaceae. Ann Bot. 2009;104(5):833-44. doi: 10.1093/aob/mcp171, PMID 19620135.
- Ali MR, Rahman MM, Ahammad KU. Effect of relative humidity, initial seed moisture content, and storage container on soybean (*Glycine max* L. Meril.) seed quality. Bangladesh J Agric Res. 2014;39(3):461-9. doi: 10.3329/bjar.v39i3.21989.
- Fatima H, Perveen A, Qamarunnisa S, Munir U. Pharmacognostic and phytochemical analyses of leaves and seed storage of *Abutilon pakistanicum* Jafri and Ali an endemic plant of Pakistan. Pak J Bot. 2014;46(6):2035-41.
- Rodrigues-Junior AG, Santos MTA, Hass J, Paschoal BSM, De-Paula OC. What kind of seed dormancy occurs in the legume genus *Cassia*? Sci Rep. 2020;10(1):12194. doi: 10.1038/s41598-020-69215-4, PMID 32699334.
- Geneve RL, Baskin CC, Baskin JM, Gehan Jayasuriya KMG, Gama-Arachchige NS. Functional morpho-anatomy of water-gap complexes in physically dormant seed. Seed Sci Res. 2018;28(3):186-91. doi: 10.1017/S0960258518000089.
- Yang Y, Jaganathan GK, Biddick M, Li J, Liu B. Identification of the water gap in *Albizia julibrissim* (Fabaceae: Mimosoideae) following wet and dry heat. Plant Biosyst an Int J Dealing Aspects Plant Biol. 2020;154(4):578-83. doi: 10.1080/11263504.2019.1651781.
- Hu D, Baskin JM, Baskin CC, Yang X, Huang Z. Ecological role of physical dormancy in seeds of *Oxytropis racemosa* in a semiarid sand land with unpredictable rainfall. J Plant Ecol. 2018;11(4):542-52. doi: 10.1093/jpe/rtx063.
- Jaganathan GK, Wu GR, Han YY, Liu BL. Role of the lens in controlling physical dormancy break and germination of *Delonix regia* (Fabaceae: Caesalpinioideae). Plant Biol (Stuttg). 2017;19(1):53-60. doi: 10.1111/plb.12451, PMID 26998975.
- Marler TE. Temperature and imbibition influence *Serianthes* seed germination behavior. Plants (Basel). 2019;8(4):107. doi: 10.3390/plants8040107, PMID 31010091.
- Fatokun K, Beckett RP, Varghese B. A comparison of water imbibition and controlled deterioration in five orthodox species. Agronomy. 2022;12(7):1486. doi: 10.3390/agronomy12071486.
- Jaganathan GK, Yule KJ, Biddick M. Determination of the water gap and the germination ecology of *Adenantha pavonina* (Fabaceae, Mimosoideae); the adaptive role of physical dormancy in mimetic seeds. AoB Plants. 2018a;10(5):ply048. doi: 10.1093/aobpla/ply048, PMID 30254728.
- Jaganathan GK. Physical dormancy alleviation and soil seed bank establishment in *Cassia roxburghii* is determined by soil microsite characteristics. Flora. 2018b;244-245:19-23. doi: 10.1016/j.flora.2018.06.004.
- Ali MR, Rahman MM, Wadud MA, Fahim AHF, Nahar MS. Effect of seed moisture content and storage container on seed viability and vigour of soybean. Bangladesh Agron J. 2018;21(1):131-41. doi: 10.3329/baj.v21i1.39392.
- Tangney R, Merritt DJ, Fontaine JB, Miller BP. Seed moisture content as a primary trait regulating the lethal temperature thresholds of seeds. J Ecol. 2019;107(3):1093-105. doi: 10.1111/1365-2745.13095.
- Li XZ, Simpson WR, Song ML, Bao GS, Niu XL, Zhang ZH, et al. Effects of seed moisture content and Epichloe endophyte on germination and physiology of *Achnatherum inebrians*. S Afr J Bot. 2020;134:407-14. doi: 10.1016/j.sajb.2020.03.022.
- Daws MI, Orr D, Burslem DFRP, Mullins CE. Effect of high temperature on chalazal plug removal and germination in *Apeiba tibourbou* Aubl. Seed Sci Technol. 2006;34(1):221-5. doi: 10.15258/sst.2006.34.1.26.
- Dell B. Structure and function of the strophilar plug in seeds of *Albizia lophantha*. Am J Bot. 1980;67(4):556-63. doi: 10.1002/j.1537-2197.1980.tb07684.x.
- Bewley JD, Black M. Seeds physiology of development and germination. 3rd ed. New York: Plenum Press; 1994.
- Burrows GE, Alden R, Robinson WA. The lens in focus-lens structure in seeds of 51 Australian Acacia species and its implications for imbibition and germination. Aust J Bot. 2018;66(5):398-413. doi: 10.1071/BT17239.
- De Paula AS, Delgado CML, Paulilo MTS, Santos M. Breaking physical dormancy of *Cassia leptophylla* and *Senna Macranthera* (Fabaceae: Caesalpinioideae) seeds: water absorption and alternating temperatures. Seed Sci Res. 2012;22(4):259-67. doi: 10.1017/S096025851200013X.
- Lersten NR 1992. Comparative morphology of the lens on *legume* (Fabaceae) seeds, with emphasis on species in subfamilies *Caesalpinioideae* and *Mimosoideae* (No. 04; USDA, QK495. L52 L4.).
- Molizane DM, Julio PGDS, Carmello-Guerreiro SM, Barbedo CJ. Physical, physiological and anatomical changes in *Erythrina speciosa* Andrews seeds from different seasons related to the dormancy degree. J Seed Sci. 2018;40(3):331-41. doi: 10.1590/2317-1545v40n3199428.
- Jayasuriya KM, Baskin JM, Geneve RL, Baskin CC. Phylogeny of seed dormancy in Convolvulaceae, subfamily Convolvuloideae (Solanales). Ann Bot. 2009;103(1):45-63. doi: 10.1093/aob/mcn217, PMID 19074450.
- Conard SG, Jaramillo AE, Cromack K Jr, Rose S. The role of the genus *Ceanothus* in western forest ecosystems. USDA Forest Serv Gen Tech Rep PNW. 1985;182.
- Baskin JM, Baskin CC, Dixon KW. Physical dormancy in the endemic Australian genus *Stylobasium*, a first report for the family *Surianaceae* (Fabales). Seed Sci Res. 2006;16(3):229-32. doi: 10.1079/SSR2006248.
- Pompelli MF, Jarma-Orozco A, Rodriguez-Páez LA. Imbibition and germination of seeds with economic and ecological interest: physical and

- biochemical factors involved. *Sustainability*. 2023;15(6):5394. doi: 10.3390/su15065394.
32. Moncaleano-Escandon J, Silva BCF, Silva SRS, Granja JAA, Alves MCJL, Pompelli MF. Germination responses of *Jatropha curcas* L. seeds to storage and aging. *Ind Crops Prod*. 2013;44:684-90. doi: 10.1016/j.indcrop.2012.08.035.
33. Hoai PTT, Tyerman SD, Schnell N, Tucker M, McGaughey SA, Qiu J, *et al*. Deciphering aquaporin regulation and roles in seed biology. *J Exp Bot*. 2020;71(6):1763-73. doi: 10.1093/jxb/erz555, PMID 32109278.
34. Corte-Real N, Endres L, Santos KPO, Figueirêdo RCB, Arruda ECP, Ulisses C, *et al*. Morphoanatomy and ontogeny of the fruit and seeds of *Jatropha curcas* L.: A promising biofuel plant. In: Segura-Campos MR, Betancur-Ancova D, editors. *The Promising Future of Jatropha curcas: proprieties and Potential Applications*. Hauppauge, NY: Nova Science Publishers, Inc; 2016. p. 141-58.

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