Short Research Article

Preliminary studies on seed dormancy of *Premna latifolia* Roxb.

Abstract

Background: The lack of understanding and consideration of dormancy and germination traits in restoration planning often contributes to plant establishment failure. It is necessary to understand the type of dormancy exist in seed of *P. latifolia* to improve seed germination and propagation techniques. **Material and Methods**: To determine the type of seed dormancy, water imbibition test and germination test were performed. **Results:** All (treated and untreated) seeds imbibed water during 4 days, however the maximum imbibition occurred in scarified seed + GA_3 500ppm for 24 h (40.3%) followed by mechanically scarified seeds (38.7%). Higher germination was observed in scarified seeds + GA_3 500ppm for 24 h (44%) followed by GA_3 500ppm for 24 h (29.3%). Whereas, lowest germination was observed in untreated seeds (6.6%). **Conclusion:** Seeds possess non -deep physiological dormancy which requires scarification and treatment to GA to enhance germination.

Keywords: *P. latifolia*, Seed Dormancy, Imbibition test, Seed germination, Gibberellic acid

Introduction:

Seed dormancy regulates germination through various physical and/or physiological means imposed by the seed coat, or within the embryo (Baskin & Baskin 2014). Dormant seeds are viable seeds that do not germinate for a period of time even under environmental conditions that are favorable for germination, and thus, they can avoid unfavorable environmental conditions (Finch Savage and Leubner-Metzger 2006). The seed germinates when the appropriate set of environmental conditions is within its range of requirements for radicle emergence, providing it has not entered secondary dormancy. Dormancy classification is based on the developmental state of the embryo at the time of seed dispersal, physical traits of the seed, and physiological responses of seeds to environmental stimuli. The dormancy-breaking requirements of different taxa can be highly specific, which seems to indicate that dormancy in general, constitute complex adaptations (Donohue et al., 2010; Linkies et al., 2010). Seed dormancy is divided into the following five categories: (1) physical dormancy (PY), (2) physiological dormancy (PD), (3) morphological dormancy (MD), (4) morpho-physiological dormancy (MPD), and (5) combinational dormancy (PY+PD) (Nikolaeva 1977;

Baskin and Baskin 1998). It is essential to be able to identify seed dormancy class in restoration practices because treatments to alleviate seed dormancy are specific to each class (Silveira 2013; Erickson et al. 2016; Kildisheva 2019). Applying the wrong treatment can result in failure to break dormancy and at worst kill the seeds. By undertaking few tests (i.e. seed quality, germination, embryo, and imbibition testing) using readily available materials, seeds of most species can be easily assigned to one of the five dormancy classes. This information is generally sufficient to inform and facilitate better seed management and restoration planning (Baskin & Baskin 2004; Hilhorst et al. 2010; Hilhorst 2011). *Premna latifolia* Roxb. Syn. *P. mollissima Roth.* is a deciduous tree belongs to family Lamiaceae. It is found distributed in the Old World tropics from Africa to China, throughout Indochina and Malaysia, to Australia and the Pacific (Harley, *et al.* 2004). All parts (leaves, root and bark) of the plant have numerous medicinal properties (Virshette, et al. 2020). The fruit is nearly a globose indehiscent drupe, 6×4 mm across, green initially, bluish-black or black upon ripening (Kumar, *et al*. 2018). According to Thapliyal and Phartyal (2005) seed of *P. latifolia* possess dormancy due to a stony endocarp that mechanically restricts protrusion of radicle. Seed germination is reported to be poor which diminishes the regeneration of the species. Thus the present investigation was conducted to determine the nature of seed dormancy of this species by the following tests: 1) comparison on the rate of imbibitions of treated and untreated seeds and 2) Effect of different treatments on seed germination.

Materials and Methods:

The experiments were carried out in Forest Tree Seed Laboratory, Forest Research Institute, Dehradun, India. For the study fully mature seeds were collected in June 2022 from 10 trees growing in kuthal village in Doon valley. Seeds were extracted from the fruits, dried, pooled and used in experiments. Seed moisture content was determined before the start of the experimentation.

Water imbibition test:

To investigate the dormancy of the seeds, water imbibition test was conducted. Three replicates of 50 seeds each were used. The dry matter of the seeds was measured, and the seeds were placed in Petri dishes with two layers of filter papers moistened with distilled water. The seeds were incubated in seed germinator (approximately 25°C ± 1). The fresh weight of seeds were measured after 6, 12, 24, 48, 72 and 96 hours of incubation. Seeds were blotted dry, weighed to nearest 0.01 g and returned to the moistened paper in the dish. Imbibition curves (increase in seed mass (fresh weight basis) over time) for each treatment were constructed and compared. The water uptake by seeds was calculated using the water uptake formula (Baskin et al. 2004).

Water absorption (%) = $[(W_2 - W_1)/W_1] \times 100$

where, W_2 is the mass of the seeds after imbibition for a given interval and W_1 is the initial seed mass.

Following treatments were given to the freshly harvested seeds-

1. Untreated seeds (Control)

2. Scarified- The endocarp was rubbed with a sandpaper on the proximal end.

3. Gibberellic Acid (GA) treated- Seeds were soaked in GA_3 solution of 500 ppm for 24 hrs.

4. Scarified + GA treated- After scarification seeds were soaked GA_3 500 ppm solution for 24 hrs.

Germination test:

Seeds were treated in the following ways for enhancement of germination:

1. Untreated- Intact seeds without any treatment

2. Seeds treated with GA: Seeds were soaked in GA_3 500 ppm for 24 hrs and sown for germination.

3. Scarification: Seeds were mechanically scarified individually by rubbing with sandpaper at the proximal end of the embryo.

4. Seeds were scarified and soaked with GA_3 500 ppm for 24 hrs before sowing. The germination test was performed by placing three replicates of 25 seeds each on moist paper in Petri dishes at 25±2 °C. The germination was monitored daily for 15 days.

Results:

Water imbibition test:

The moisture content of freshly collected seed was 11.8%. In the imbibition test, the mass of untreated, scarified, GA_3 500ppm for 24 h and scarified seed + GA_3 500ppm for 24 h increased by approximately 3%, 11%, 3% and 11% after 6 hours of imbibition and 29%, 39%, 30% and 40% after 96 hours of imbibition respectively. All (treated and untreated) seeds imbibed water during 4 days, however the maximum imbibition occurred in mechanically scarified seeds (38.7%) and scarified seed + GA_3 500ppm for 24 h (40.3%).

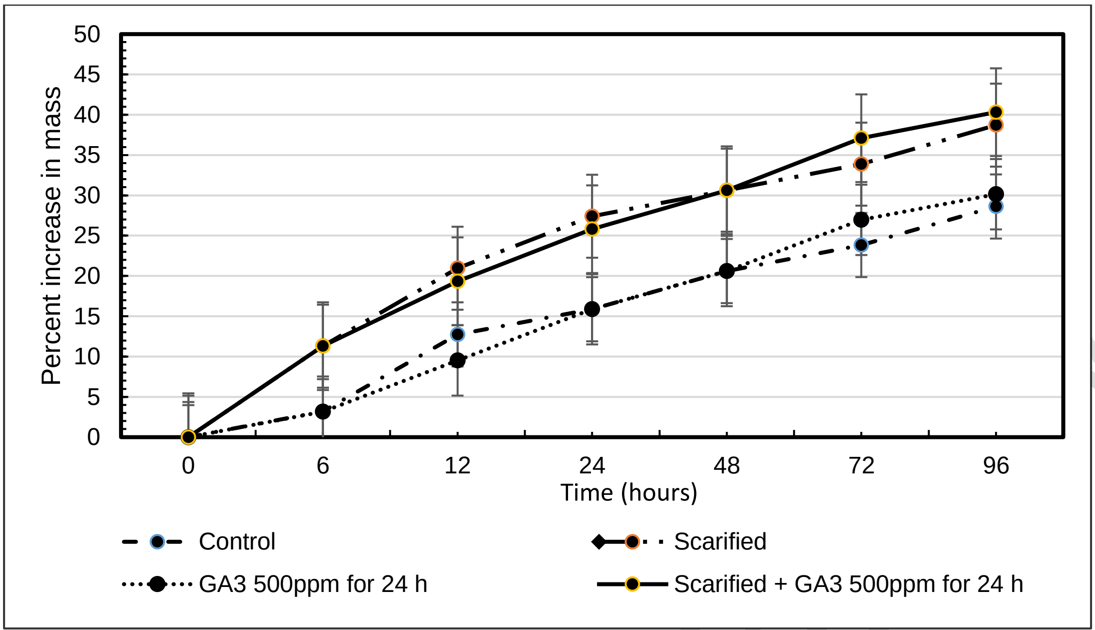

Figure 1. Percentage increase in fresh weight of untreated and treated seeds of *Premna latifolia* Roxb. imbibed on filter paper. Error bars represent ± standard error]

Germination test:

In the study, different treatments had significant effect on seed germination. Higher germination was observed in scarified seeds + GA_3 500ppm for 24 h (44%) followed by GA_3 500ppm for 24 h (29.3%). Whereas, lowest germination was observed in untreated seeds (6.6%).

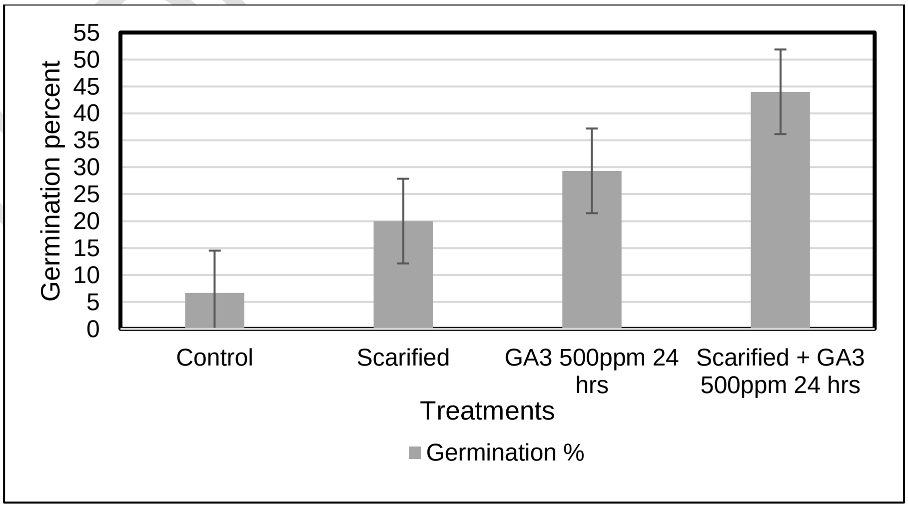

Figure 2. Mean germination percentage (±standard error) of untreated and treated seeds of *P. latifolia* Roxb.

Discussion:

Baskin and Baskin (1998) categorized physiological dormancy in three types- (1) Deep- Excised embryo produces abnormal seedling, GA does not promote germination and seeds require 3–4 months of cold stratification to germinate, (2) Intermediate- Excised embryo produces normal seedling, GA promotes germination in some (but not all) species, seeds require 2–3 months of cold stratification for dormancy break and dry storage can shorten the cold stratification period (3) Non deep- excised embryo produces normal seedling, GA promotes germination, depending on species, cold (0–10°C) or warm (15°C) stratification breaks dormancy, seeds may after-ripen in dry storage and scarification may promote germination. Non-deep physiological dormancy is prevalent in the family Lamiaceae and this type of dormancy is reported to be broken during dry storage at room temperature (i.e. after-ripening) (Baskin and Baskin, 2014, Baskin and Baskin, 2020, Holdsworth et al., 2008). According to Baskin and Baskin (2003), if the mass of seeds increases to ≤ 20% in the water imbibition test, the seeds are considered impermeable to water. In the present study, the seeds of *P. latifolia* imbibed water readily irrespective of given treatment. Seed coat found to be a physical barrier in absorption of water to some extent however, seed scarification increased water absorption. Germination was also observed higher in scarified seeds in combination with GA treatment as compared to separate treatment. Thus the study suggests species have non-deep physiological dormancy. *Schinus molle* L. is reported to have physiological dormancy in which, both scarified and non- scarified seeds absorbed water in imbibition test and treatment of H2SO4 after 3 months of dry storage improved germination (Pereira *et al*. 2016). Kundu and Chaturvedi, (2019) also reported that combined treatment of scarification and GA application increased the germination to 72.6% in *Schleichera oleosa* seeds which suggested that the species has combinational dormancy i.e. Physical and Physiological.

Conclusions

The present study revealed that in the imbibition test, seeds of *P. latifolia* can absorb water readily. Seed has stony endocarp which restricts absorption of water to some extent however, scarification of seed can increase imbibition of water. Germination was also increased when seeds were treated with gibberellic acid after scarification. This indicates that seeds have low growth potential which can be enhanced by application of GA. Thus it is concluded that seeds of *P. latifolia* have non-deep physiological dormancy.

Reference:

1. Baskin C.C. and Baskin J.M. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination, 2nd Edition, Academic/Elsevier, San Diego, CA, USA. 2014.
2. Baskin CC, Baskin JM. Seeds: ecology, biogeography, and, evolution of dormancy and germination. San Diego: Academic; 1998.
3. Baskin JM, Baskin CC. A classification system for seed dormancy. Seed Sci Res. 2004;14:1–16.
4. Baskin, C. C., & Baskin, J. M. Breaking seed dormancy during dry storage: a useful tool or major problem for successful restoration via direct seeding?. Plants, 2020; 9(5), 636.
5. Donohue K, Rubio de Casas R, Burghardt L, Kovach K, Willis CG. Germination, postgermination adaptation, and species ecological ranges. Annual Review of Ecology, Evolution and Systematics. 2010; 41: 293–319.
6. Erickson TE, Barrett RL, Merritt DJ, Dixon KW. Pilbara seed atlas and field guide: plant restoration in Australia’s arid northwest. CSIRO Publishing, Dickson, Australian Capital Territory, Australia. 2016.
7. Finch-Savage WE, Leubner-Metzger G. Seed dormancy and the control of germination. New Phytologist. 2006;171: 501–523.
8. Harley R M, Atkins S, Budantsev A L, Cantino P D, Conn B J, Grayer R, Harley M M, Kok, R P J de, Krestovskaja T, Morales R, Paton A J, Ryding O and Upson, T. Labiatae In: Kubitzki, K. (ed) The families and genera of vascular plants VI, Berlin: Springer, 2004; pp. 167–275.
9. Hilhorst HWM. Standardizing seed dormancy research. Pages 43–52. In: Kermode A (ed) Seed dormancy: methods and protocols. Humana Press, Totova, NJ
10. Hilhorst HWM, Finch-Savage WE, Buitink J, Bolingue W, Leubner-Metzger G. Dormancy in plant seeds. Topics in Current Genetics. 2011;21:43–67
11. Holdsworth, M. J., Bentsink, L., & Soppe, W. J. Molecular networks regulating Arabidopsis seed maturation, after-ripening, dormancy and germination. New phytologist, 2008; 179(1), 33-54.
12. Kildisheva OA. Improving the outcomes of seed-based restoration in cold and hot deserts: an investigation into seed dormancy, germination, and seed enhancement. PhD Dissertation, University of Western Australia, Perth. 2019. https://doi.org/10.26182/5caea3c9861f2

13. Kumar B D, Deepika D S and Raju S. On the reproductive ecology of Premna latifolia L. and Premna tomentosa willd. (Lamiaceae). Journal of Threatened taxa. 2018; 10(1):11105-11125. DOI: httpdoi.org10.11609jott.3201.10.1.11105-11125
14. Kundu, M., & Chaturvedi, N. Preliminary studies on seed dormancy of Schleichera oleosa (Lour.) Merr. Trop. Plant Res, 2019; 6:133-138.
15. Linkies A, Graeber K, Knight C, Leubner-metzger G. The evolution of seeds. New Phytologist 2010; 186: 817–831.
16. Nikolaeva MG. Factors controlling the seed dormancy pattern. In: The physiology and biochemistry of seed dormancy and germination (ed. A. A. Khan). Amsterdam: North-Holland Publishing. 1977. p. 51–74.
17. Pereira, M. P., Corrêa, F. F., Polo, M., de Castro, E. M., Cardoso, A. Á., & Pereira, F. J. Seed germination of Schinus molle L.(Anacardiaceae) as related to its anatomy and dormancy alleviation. Seed Science Research, 2016; 26(4), 351-361
18. Silveira FAO. Sowing seeds for the future: the need for establishing protocols for the study of seed dormancy. Acta Botanica Brasilica 2013;27: 264–269
19. Thapliyal R.C. and Phartyal, S.S. Dispersal and germination syndromes of tree seeds in a monsoonal forest in northern India. Seed Science Research. 2005; 15:29–42.
20. Virshette S J, Patil M K and Shaikh J R. A review on pharmacological properties and phytoconstituents of indigenous carminative agents. Journal of Pharmacognosy and Phytochemistry. 2020; 9(3): 142-145.