Short Research Article

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

Abstract

Background: *Premna latifolia* Roxb. (Lamiaceae) is a deciduous tree distributed in Africa to China, throughout Indochina and Malaysia, to Australia and the Pacific. The various parts of the plant (leaves, stem bark and root) is reported to have numerous medicinal properties. The lack of understanding and consideration of dormancy and germination traits of a species could result in unsuccessful restoration programme. The aim of the present study was to investigate the nature of seed dormancy of *P. latifolia* that have low germination in natural conditions. **Material and Methods**: To determine the type of seed dormancy, water imbibition and germination test were performed. Seeds were treated by mechanical scarification, Gibberellic acid (GA) and combined treatment of scarification and GA before allowing them to imbibe in moist paper. **Results:** All the treated (mechanically scarified, GA treated, mechanically scarified + GA treated) and untreated (control) seeds imbibed water for 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

1. Introduction:

Seed dormancy regulates germination through various physical and/or physiological means imposed by the seed coat, or within the embryo (Baskin and 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 and Baskin, 2004; Hilhorst et al., 2010; Hilhorst, 2011). *Premna latifolia* Roxb. Syn. *P. mollissima* Roth. is a deciduous tree belonging 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). Various studies on phytochemical composition of *P. latifolia* have reported the presence of active ingredients such as glycosides, steroids, saponins, phenols, flavonoids, terpenoids, and tannins (Ruwali and Negi, 2019). All parts (leaves, root and bark) of the plant have numerous medicinal properties (Virshette et al., 2020). Roots are appetizer, astringent, useful in abscess, asthma, bronchitis, cardiac disorders, cough, diabetes, diarrhoea, inflammations, neuralgia, obesity, rheumatoid arthritis, rhinitis and used in ayurvedic preparations such as amritarista, dantyadyarista, dasamularista, cyavanaprasa etc. (AFI, 2007; Dianita and Jantan, 2017; Virshette et al., 2020). The leaves of *P. latifolia* Roxb. are diuretic, useful in agalactia, allergy, colic, cough, dropsy, dyspepsia, flatulence, neuralgia, piles, rheumatalgia and tumours (Quattrocchi, 2012). The stem bark is applied to heal wounds, eczema, ring-worms, boils, skin diseases, itches and to reduce fever (Kumari et al., 2013). 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 imbibition of treated and untreated seeds and 2) effect of different treatments on seed germination.

2. Materials and Methods:

The experiments were carried out in Forest Tree Seed Laboratory, Forest Research Institute, Dehradun, India. Fully mature seeds were collected from 10 trees with abundant fruiting from "Kuthal" village in Doon valley at an altitude of 970msl (latitude/longitude 30°24'20.73"N/78°05'01.70"E) in June 2022. Seeds were extracted from the fruits, dried, pooled and used in experiments as explained below. Four replications of 50 seeds were used to calculate moisture content on a fresh weight basis after drying the seed samples at 103±2℃ in a hot -air oven for 17h (ISTA, 2010). To determine the nature of dormancy two experiments were conducted.

2.1 Water imbibition test:

To investigate the dormancy of the seeds, water imbibition test was conducted. Three replicates of 50 seeds each were used. Prior to the imbibition test, the dry matter of the seeds was measured and recorded. Thereafter, seeds were placed in Petri dishes with two layers of moistened filter papers and incubated in seed germinator (approximately 25°C ± 1). "The weight of seeds was 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 petridishes. 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.

The following treatments were given to the freshly harvested seeds-

1. Untreated seeds (Control)- seeds were kept in filter paper moistened with distilled water.

2. Scarified- The endocarp was rubbed with a sandpaper on the proximal end and kept in filter paper moistened with distilled water.

3. Gibberellic Acid (GA) treated- Seeds were kept in filter paper moistened with GA_3 500 ppm solution.

4. Scarified + GA treated- After scarification seeds were kept in filter paper moistened with GA_3 500 ppm solution.

2.2 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 till thinning of the endocarp.

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 (ISTA, 2010). The germination was monitored daily for 15 days.

2.3 Statistical analysis

Statistical analysis was done using two way analysis of variance (ANOVA) using SPSS software. Tukey's HSD was used to determine significant difference among treatment means at 5% level of significance.

3. Results

3.1 Water imbibition test

The average moisture content of seed samples 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% respectively, after 6 h of imbibition test. After 96 h, percent increase in mass reached to 29% (untreated), 39% (scarified), 30% (GA_3 500ppm for 24 h) and 40% (scarified seed + GA_3 500ppm for 24 h). All the treated (mechanically scarified, GA treated, mechanically scarified + GA treated) and untreated (control) seeds imbibed water for 4 days, however the maximum imbibition occurred in mechanically scarified seeds (38.7%) and scarified seed + GA_3 500ppm for 24 h (40.3%).

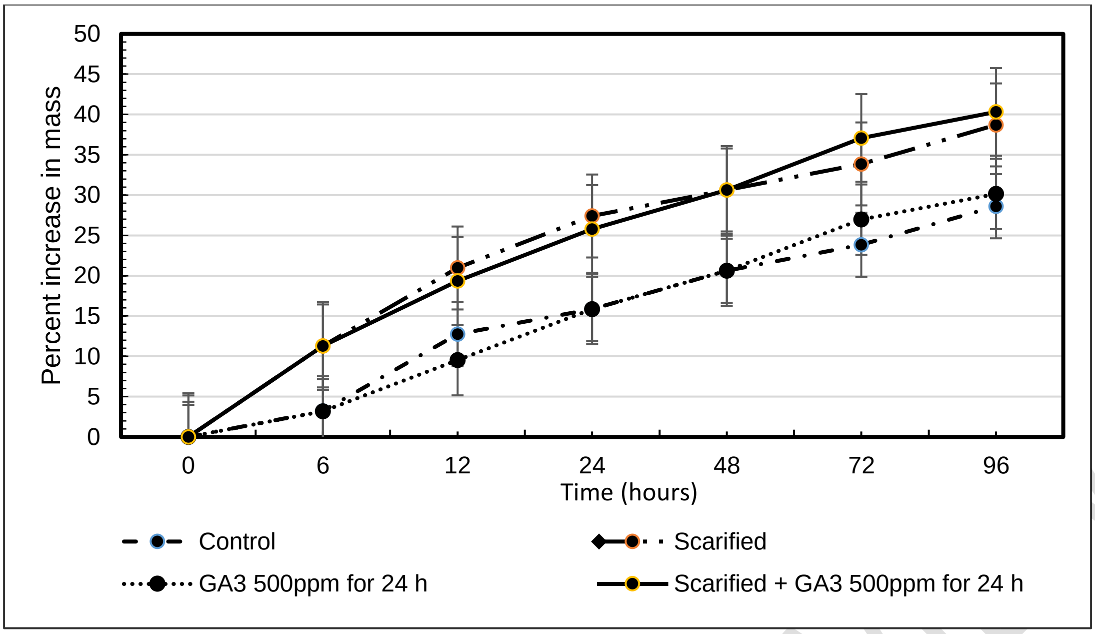

Figure 1. Percentage increase in fresh weight of untreated and treated seeds of *Premna latifolia* Roxb. imbibed water. Error bars represent ± standard error

3.2 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%).

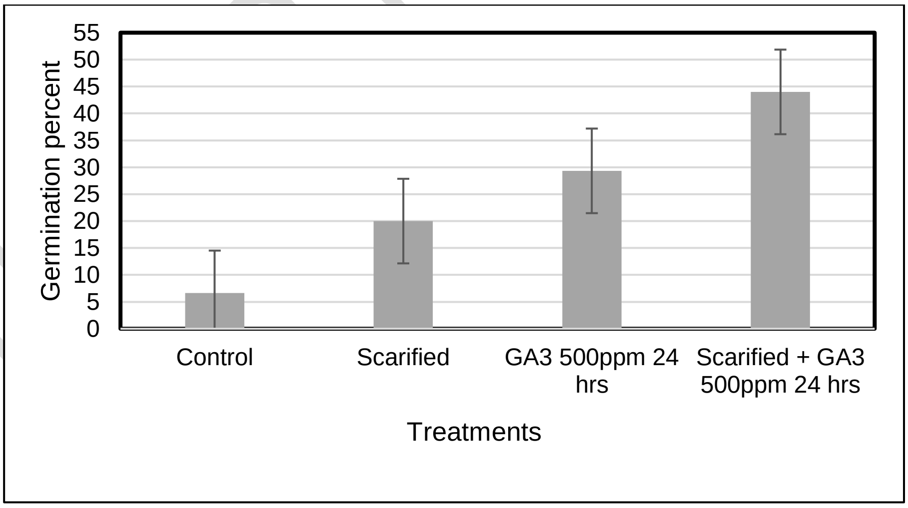

Figure 2. Mean germination percentage (±standard error) of seeds of *P. latifolia* Roxb.

4. 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 to germinate 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 H_2SO_4 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* (Lour.) Merr. seeds which suggested that the species has combinational dormancy (i.e. Physical and Physiological).

5. Conclusion

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.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Reference

1. Baskin C.C. and 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.
2. 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.
3. Baskin C.C. and Baskin J.M. Seeds: ecology, biogeography, and, evolution of dormancy and germination. San Diego: Academic; 1998.
4. Baskin J.M. and Baskin C.C. A classification system for seed dormancy. *Seed Sci Res*. 2004;14(1):16.
5. Dianita R. and Jantan I. Ethnomedicinal uses, phytochemistry and pharmacological aspects of the genus *Premna*: a review. *Pharmaceutical Biology*. 2017;55(1):1715–1739. DOI: 10.1080/13880209.2017.1323225
6. Donohue K., Rubio de Casas R., Burghardt L., Kovach K. and Willis C.G. Germination, post germination adaptation, and species ecological ranges. *Annual Review of Ecology*, Evolution and Systematics. 2010;41:293–319.
7. Erickson T.E., Barrett R.L., Merritt D.J. and Dixon K.W. Pilbara seed atlas and field guide: plant restoration in Australia's arid northwest. CSIRO Publishing, Dickson, Australian Capital Territory, Australia. 2016.
8. Finch-Savage W.E. and Leubner-Metzger G. Seed dormancy and the control of germination. *New Phytologist*. 2006;171:501–523.
9. 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;167–275pp.
10. Hilhorst H.W.M. Standardizing seed dormancy research. *Methods Mol Biol*. 2011;773:43-52. doi: 10.1007/978-1-61779-231-1_3.
11. Hilhorst H.W.M., Finch-Savage W.E., Buitink J., Bolingue W. and Leubner-Metzger G. Dormancy in plant seeds. *Topics in Current Genetics*. 2011;21:43–67.

12. Holdsworth M.J., Bentsink L., and Soppe W.J. Molecular networks regulating Arabidopsis seed maturation, after-ripening, dormancy and germination. *New phytologist*, 2008;179(1):33-54.
13. International rules for seed testing (ISTA). International Seed Testing Association (ISTA) Zurich, Switzerland. 2010.
14. Kildisheva O.A. 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
15. 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
16. Kumari H., Shrikanth P., Pushpan R., Harisha C.R. and Nishteswar K. Comparative pharmacognostical evaluation of leaves of regionally accepted source plants of agnimantha. *J. Res. Educ. Indian Med*. 2013;19(1-2):1-8.
17. Kundu M. and Chaturvedi N. Preliminary studies on seed dormancy of *Schleichera oleosa* (Lour.) Merr. *Trop. Plant Res*. 2019;6:133-138.
18. Linkies A., Graeber K., Knight C. and Leubner-metzger G. The evolution of seeds. *New Phytologist*. 2010;186:817–831.
19. Nikolaeva M.G. 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;51–74pp.
20. Pereira M.P., Corrêa F.F., Polo M., de Castro E.M., Cardoso A.Á., and 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.
21. Quattrocchi U. CRC world dictionary of medicinal and poisonous plants: common names, scientific names, eponyms, synonyms, and etymology. CRC Press, Boca Raton, Florida. 2012;869-870.
22. Ruwali P. and Negi D. Phytochemical analysis and evaluation of antioxidant activity of *Premna latifolia* Roxb. A medicinal plant (Family: Lamiaceae). *The Pharma Innovation Journal*. 2019;8(5):13-20.
23. Silveira F.A.O. Sowing seeds for the future: the need for establishing protocols for the study of seed dormancy. *Acta Botanica Brasilica*. 2013;27:264–269.

24. 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.
25. The Ayurvedic Pharmacopoeia of India, Formulations. Part II. 1st ed., Vol. I, 13-16. New Delhi: Government of India, Ministry of Health and Family Welfare, Department of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy (AYUSH); 2007;65-70pp.
26. 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.