



Research article

Preliminary studies on seed dormancy of *Schleichera oleosa* (Lour.) Merr.

Maitreyee Kundu* and Nimisha Chaturvedi

Tropical Forest Research Institute, P.O. R.F.R.C., Mandla Road, Jabalpur-482021, Madhya Pradesh, India

*Corresponding Author: spalliwest@yahoo.co.in

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Abstract: *Schleichera oleosa* is a tropical deciduous tree species distributed in south-east Asia. The aim of the present study was to investigate the nature of seed dormancy of *Schleichera oleosa* that have low germination under laboratory conditions. Seeds were treated by scarification, Gibberellic acid (GA), and combined treatment of scarification and GA before allowing them to imbibe in moist paper. Maximum water uptake was observed in seeds that were soaked in GA after scarification. The moisture content of seed coat remained unchanged during imbibition that suggests the water-impermeable nature of seed coat. Scarification accelerated germination, but it could not fully eliminate dormancy. The highest germination was found in the combined treatment of scarification and GA application. Dry storage at room temperature for 9 months broke dormancy and allowed germination of untreated seeds at 28°C. The results indicate that *Schleichera oleosa* seeds exhibit both physical (for its water-impermeable seed coat) and physiological dormancy and need afterripening for 9 months to overcome the dormancy. The ecological perspective of dormancy of this tropical seed has been discussed.

Keywords: Dormancy - Imbibition - Seed pretreatment - Dry storage - Germination - *Schleichera oleosa*.

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INTRODUCTION

Dormancy is protective mechanism of plant against unfavourable environment. It helps in successful establishment and maintaining the plant population in natural environment. It is a complex heritable trait regulated by plant growth hormones, affected by developmental and environmental factors before and after the seed is formed (Koornneef *et al.* 2002, Finch-Savage & Leubner-Metzger 2006). After shedding, break in dormancy is largely dependent on the environmental factor that enables the maximum number of seeds to germinate in favourable conditions. Nature of dormancy in temperate species is well known, as huge number of work have been published on seed dormancy of those species. On the other hand, limited knowledge in dormancy and germination of tropical species especially in *in-situ* conditions is the main constraint of the restoration program of the tropical forest that have already been disturbed significantly due to human interference. It is well-known that most of the tropical species are non-dormant (Casasola 1976). This may be attributed to the fact that seeds are programmed to mature in favourable season for their germination (*i.e.* temperature and water availability). In spite of that some species are also remain dormant (Ng 1973) and the cause and break of dormancy in natural environment is not known.

Schleichera oleosa (Lour.) Merr. belongs to the family Sapindaceae. Members of this family are distributed in vast ecological conditions. Species of this family mostly have physical dormancy (Cook *et al.* 2010), others have physiological or combined dormancy (Baskin & Baskin 2004). In Indian subcontinent, *Sapindus mukorossi* Gaertn. had been reported to have physical dormancy and *S. trifoliatius* L. have physical and physiological dormancy (Baskin & Baskin 2004). Radhamani *et al.* (2003) observed better germination in *Schleichera oleosa* seeds by breaking of seed coat and soaking overnight in water followed by Gibberellic Acid (GA₃) and opined that the seed had seed coat dormancy. Thapliyal & Tewari (2011) also concluded that mechanical scarification resulted maximum germination of *S. oleosa* seeds. They also observed after-ripening for 2 months could increase the germination percentage. However, it is not known how germination of this species is regulated in

tropical deciduous forest having precipitation during monsoon season and the nature of dormancy was not fully understood.

The purpose of this present study is to reveal 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 change in moisture content of seed coat and cotyledons after soaking; 2) effect of different pretreatments on germination of seeds and 3) effect of dry storage on germination capacity of seed.

MATERIALS AND METHODS

Scheleichera oleosa (Lour.) Merr. (family: Sapindaceae) occurs from the foothills of the Himalayas and the western Deccan of India to Sri Lanka and China. It spreads spontaneously in dry deciduous forest and savannas with an altitude up to 1200 m having normal rainfall of 750 to 2800 mm. The absolute maximum temperature is 35–40°C and minimum being 2–4°C. Seeds are subglobular, about 12 mm × 10 mm × 8 mm; hilum is orbicular, testa is brown, smooth, glabrous enclosed in a succulent yellow aril. Number of seeds per kg varies from 1400 to 2200 kg⁻¹. It is an economically important plant, work as best host for the lac insect (*Laccifer lacca*). The pinkish brown heartwood is very hard and durable, excellent to make cartwheels, tool handles, roller for sugar mills etc. Fruits are edible and oil from seed is used for culinary purposes, lighting and preparing traditional medicines. *S. oleosa* is deciduous, but completely leafless for a few days only. Leaves drop in December to February. The racemes of greenish yellow flowers appear with the young leaves in February–April (Chaudhary *et al.* 2016). The fruits fall quickly to the ground after they ripen in June–August.

The experiments were carried out in the laboratory of Seed Technology, Tropical Forest Research Institute, Jabalpur in India. Fully mature seeds were collected from 10 trees of *Scheleichera oleosa* growing in the Mandla District of Madhya Pradesh in India. Seeds from this mass collection were extracted from the fruits, dried, pooled and used in experiments with 15 days of collection. Seed moisture content was determined before the start of the experimentation.

To determine the nature of dormancy three experiments were conducted:

Imbibition test: Four treatments were given in freshly harvested seeds,

1. Untreated seeds were soaked in water at ambient (27–30 °C) temperature for 24 hrs.
2. Scarified- The pericarp was nicked with a scalpel on the proximal end and soaked in water at ambient temperature for 24 hrs.
3. Gibberellic Acid (GA) treated- Seeds were soaked in GA₃ solution at the dose of 500 ppm for 24 hrs.
4. Scarified + GA treated- After scarification seeds were soaked GA₃ 500 ppm solution for 24 hrs.

The initial dry mass of three 25 seed replicates was measured. After treatment, the surfaces of the seeds were blotted dry with a paper towel and fresh weight was determined. This was considered as 0 hrs. Seeds were then placed into dishes between two sheets of moistened blotting paper-towel. The experiment was conducted at ambient room conditions (27–30 °C). After 12, 24, 48, 72, 96, 144 hrs, seeds were removed from the Petri dishes, 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.

Water uptake by the seed parts

To determine the water uptake, another two sets of 100 seeds were placed between blotted paper sheets after following treatments: 1) scarified (individually with a single-edge razor blade) 2) scarified + GA- treated 3) GA treated and of 4) untreated seeds. Sampling was done after fixed intervals for three replications of fifty seeds. During each sampling, surface water of seeds (with and without seed coat) was dried up with blotting paper and moisture contents of seed coat, cotyledon and intact seed were estimated by drying the entire seeds and their parts separately at 103°C for 17 hrs. Moisture content was estimated as percentage of fresh weight.

Germination

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 GA₃ 500 ppm for 17 hrs and sown for germination.
3. Scarification: Seeds were mechanically scarified individually with a single-edge razor blade at the distal end of the embryo.
4. Seeds were scarified and soaked with GA₃ 500 ppm for 17 hrs before sowing.

The germination test was performed by placing three replicates of 50 seeds each on moist paper in Petri dishes at 28±2 °C in darkness. The germination was counted daily and seeds were considered to be germinated

when the radicles grew at least 1 cm. The number of rotten, empty, good germinated and un-germinated seeds was determined by cutting test after 30 days of sowing. Germination percentage was calculated from this data.

Effect of dry storage on dormancy

Freshly harvested seeds were stored at temperature sequences simulating temperatures that seeds experience in nature according to the season (varies 10–40 °C) during the month of July 2011. Germination was assessed 0, 3, 6, 9 and 12 months after collection to note the change in germination with time. Besides untreated seeds, two treatments were given to the seeds: scarification and scarification with GA₃ 500 ppm before germination test. Three replicates of 50 seeds in each treatment were placed at 28±2 °C in darkness.

Data were analysed statistically using two-way analysis of variance (ANOVA) using SPSS software. Fisher's least significant difference test was used to determine significant differences ($P < 0.05$) between individual treatments.

RESULTS

Effect of different treatments on rate of water uptake

There was little increase in seed weight for non-treated seeds (Fig. 1). Only about 5% increase was observed during 7 days in imbibed condition. 17.4% increase in mean was observed when the seeds were exposed to scarification treatment. However, combined treatment of mechanical scarification and GA caused maximum subsequent water uptake (about 45%). This treatment caused significant increase in mass within three days on moist filter paper at room temperature in comparison to other treatments.

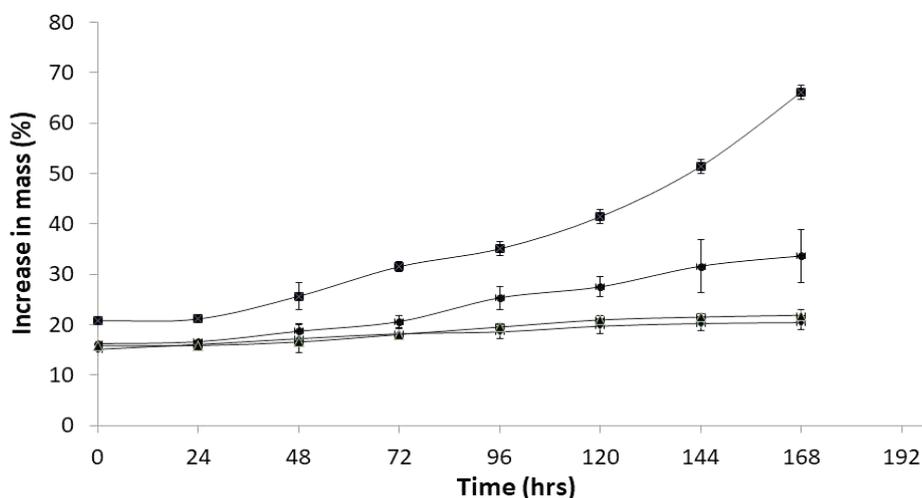


Figure 1. Percentage increase in fresh weight of untreated and treated seeds of *Schleicheria oleosa* (Lour.) Merr. imbibed on germination blotter. [-♦-, Untreated; -●-, Scarification; -▲-, GA treatment; -■-, Scarification with GA treatment; Error bars represent ± standard error]

Effect of different treatment on moisture contents of seed coat and cotyledon

Fig. 2 shows that no significant changes were observed in moisture content of seed coat in all treatments. Moisture content of cotyledon was not changed in untreated and GA treated seeds. Scarification helped in increase in moisture content of some seeds. Significant increase in moisture content was observed in the scarified seeds treated with GA. Seeds with this combined treatment, water uptake started within one day and moisture content of cotyledon increased to 29.4% and 35.2% after 4 and 8 days respectively.

Effect of different treatments on germination percentage

Mechanically scarified seeds treated with gibberellic acid (GA₃) at 500 ppm showed highest germination of about 72.6%. Scarified seeds those were scarified, but not treated with GA achieved 29.6% germination. Seeds soaked in GA 500 ppm showed only 11.5% germination whereas untreated seeds (not treated) had 5.8% germination (Fig. 3).

Effect of dry storage on germination

Significant increase in germination was observed after 9 months of storage in all treated and untreated seeds (Table 1). Though scarification caused increase in germination percentage, negligible change in germination was observed in untreated and scarified seeds till 6 months of storage. Combined treatment of scarification and soaking in GA showed the maximum germination of freshly harvested seeds, as did 9 months storage of untreated seeds.

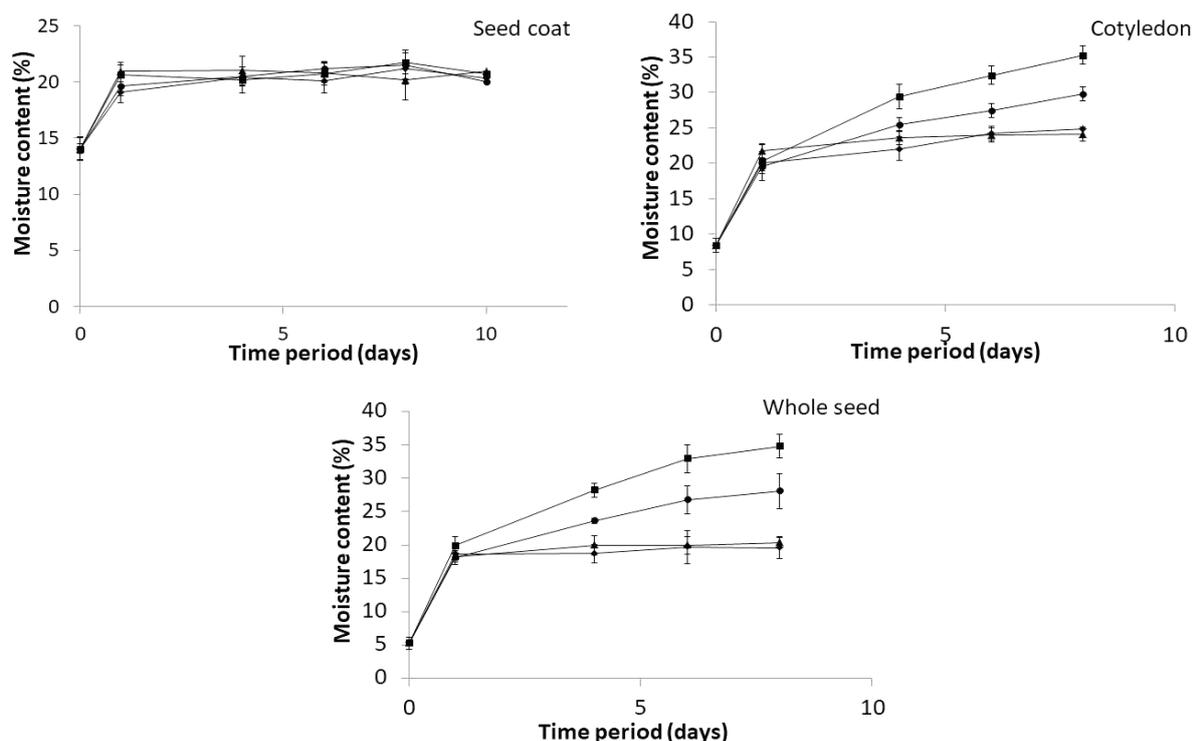


Figure 2. Changes in moisture content of seed coat, cotyledon and whole seed in untreated and treated seeds of *Schleichera oleosa* (Lour.) Merr. [-♦-, Untreated; -●-, Scarification; -▲-, GA treatment; -■-, Scarification with GA treatment; Error bars represent ± standard error]

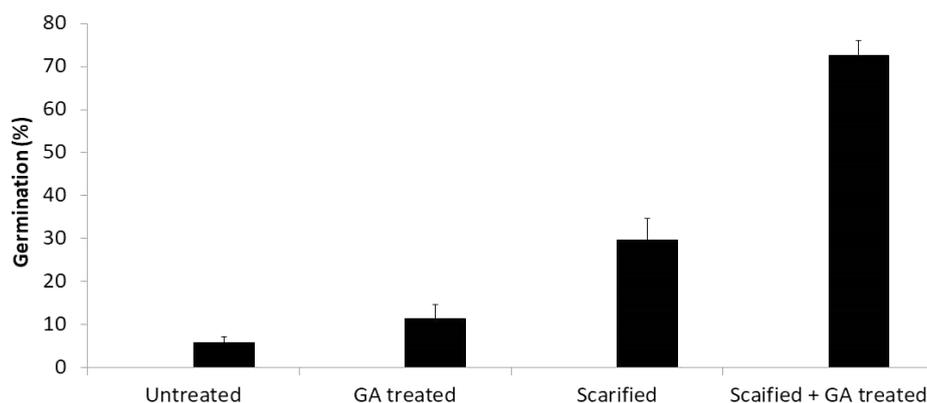


Figure 3. Mean germination percentage (±standard error) of untreated and treated seeds of *Schleichera oleosa* (Lour.) Merr.

Table 1. Mean germination percentage of untreated and treated seeds of *Schleichera oleosa* (Lour.) Merr. after different periods of dry storage.

Months of storage	Treatments		
	Untreated	Scarified	Scarified+ GA
0	7.3 ^a	35.3 ^a	70.7 ^a
3	6.0 ^a	27.3 ^a	56.0 ^a
6	8.0 ^a	32.7 ^a	66.0 ^a
9	74.0 ^b	79.3 ^b	72.7 ^a
12	79.3 ^b	74.0 ^b	67.3 ^a

Note: Different letters in the same column denote significant differences (P<0.05) using LSD.

DISCUSSION

The imbibitions experiment demonstrated that the untreated seed of *Schleichera oleosa* imbibed very low amount of water, whereas water uptake was more in scarified seeds than untreated seeds. The seed coat was impermeable to water as was evident from the experiment 3 where moisture content of seed coat remained unchanged in all of the four treatments including untreated one. Germination percentage of scarified seeds was improved to some extent. This clearly indicates that seed coat imposed physical barrier in water absorption. Though mechanical scarification can promote germination in physiological dormancy, imbibitions study can be a best method to identify physical dormancy (Baskin & Baskin 2004). More increase in fresh weight was

observed in scarified in comparison to unscarified seeds during imbibitions in summer farewell [*Dalea pinnata* (J.F.Gmel.) Barneby], a species with physical dormancy (Perez *et al.* 2009). About 75% increase in fresh weight was noted in seeds scarified with hot water in six physically dormant genera of Rhamnaceae, compared with non-treated seeds having less than 16% increase in fresh weight. Apart from scarification, GA plays an important role in germination of *Schleichera oleosa* seeds. Significant uptake of water was observed in scarified seeds treated with GA, whereas germination percent was very low in when the seeds were treated with GA only. Therefore, GA was not absorbed due to impermeability of seed coat of freshly collected seeds. Also, combined treatment of scarification and GA application had increased the germination to 72.6%. The result suggests that this species has two types dormancy *i.e.* physical and physiological. Similar nature of dormancy was observed in *Koelreuteria paniculata* Laxm. (Park & Rehman 1999) and *Sapindus drummondii* Hook. & Arn. (Munson 1984), two other members of the Sapindaceae. Cirak *et al.* (2004) observed combined treatment of GA and H₂SO₄ increased the germination of black henbane (*Hyoscyamus niger* L.) seeds and concluded that the seeds have double dormancy involving a hard seed coat and a partially dormant embryo. On the other hand, Pereira *et al.* (2016) concluded that seeds of *Schinus molle* L. had physiological dormancy though maximum germination occurred in the seeds those were treated with H₂SO₄ after 3 months of dry storage. They observed both scarified and non-scarified seeds absorbed water in imbibition test.

Effect on dry storage on germination showed that no remarkable change in germination was observed till 6 months of storage. After 9 months of dry storage seeds achieved full germination capacity without any treatment. It indicates that seeds have physiological dormancy in addition to physical dormancy. Fresh seeds need scarification for absorption of water, for this reason they could not absorb GA that resulted very few germination of GA treated fresh seeds. However, after-ripening or dry storage for 9 months helped to remove the dormancy in such a way that enabled the seeds in absorption of water through the water-gap (Gama-Arachchige *et al.* 2011) and growth of the embryo. Imbibition test and water uptake by seed parts after sowing supports presence of physical dormancy in the seeds of this species. Thapliyal & Tewari (2011) also observed maximum germination of *Schleichera oleosa* seeds which were after-ripened and mechanically scarified. Dormancy due to impermeability of seed coat and dormant embryo had been termed as combinational dormancy (Baskin & Baskin 1998).

Dry storage acts an environmental cue for break of physical and physiological dormancy in several species including Sapindaceae (Turner 2005) in temperate zones though it was not documented in tropical species. Seed fall in *Schleichera oleosa* occurs during July–August (after the start of the monsoon rain). After this period temperature starts to fall and dry period starts which are not favourable for seedling growth. Combined dormancy in *Schleichera oleosa* acts a safety mechanism for survival that prevents the seeds to germinate in the first season and provide protection for survival up to the next season. It is also important to note that seeds were able to overcome dormancy at the commencement of summer months (2–3 months before rain) to get the maximum benefit of rain for germination and consecutive seedling survival. The combination of hard seed coat and dormant embryo was reported in arid regions and mediterranean climate that protects the seed from germinating after out of season rains (Kigel 1995, Norman *et al.* 1998). Completion of seed maturation just before or during monsoon is common feature of dry deciduous forests of Indian subcontinent and seeds maturing during or just before rain do not generally possess dormancy. However the arrival of monsoon differs in different region and rain does not appear at the same time in each year. Seeds do not get ample moisture for germination and seedling survival. Also moisture requirement for growth and tolerance to desiccation varies among species. So, development of dormancy appears to be a protective mechanism for their better survival.

The factors that break physical and physiological dormancy may vary. In *Schleichera oleosa* few fresh seeds germinated in control environment (dark 28°C) and germination percentage were not increased till 6m of storage in the laboratory condition that imitate seasonal temperature and humidity. After 9 months of storage, growth potential of the embryo of untreated seeds increased and cells of the water gap in the seed coat became weak that resulted in radical protrusion or germination. It appears that change in temperature and humidity during autumn, winter and spring was responsible for removal of dormancy in this species.

It was observed that the seeds of this species could germinate at fixed range of temperature of 25–40 °C and unlike temperate species change in germination temperature was not able to break the dormancy (Kundu, unpublished manuscript). But storage temperature could be an important factor for break of dormancy of tropical species. Argel & Patson (1999) opined that it was an important factor for natural softening of seed coat. The future work on storage temperature on dormancy break may indicate the factors working in dormancy-breaking mechanism of the seeds of this species.

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