

After-ripening, Light Conditions, and Cold Stratification Influence Germination of Marula [*Sclerocarya birrea* (A. Rich.) Hochst. subsp. *caffra* (Sond.) Kokwaro] Seeds

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Abstract. Marula [*Sclerocarya birrea* (A. Rich.) Hochst. subsp. *caffra* (Sond.) Kokwaro (Anacardiaceae)] is used in many African countries as a food crop and is also in demand for industrial purposes. The fruit pulp has high vitamin C levels and the nuts have a high protein and oil content. The fruit pulp is commercially used in the production of an alcoholic beverage (Amarula Cream) and the oil is gaining importance in the cosmetic industry. Although attempts are being made to domesticate this high-value indigenous tree, there is very limited information available on aspects of seed germination. Our study investigated the role of light, temperature, cold stratification, and after-ripening on seed germination of *S. birrea*. Temperatures between 25 and 35 °C favored germination of opercula-removed seeds under continuous dark conditions. White light completely inhibited seed germination with the inhibitory effect being reversed when seeds were transferred to dark conditions. This photoinhibitory effect on opercula-removed seeds was lost after 12 months of seed storage at room temperature in the dark. Cold stratification (5 °C) of intact seeds for 14 days significantly improved germination (65%) as compared with nonstratified seeds (32%). Pregermination treatments (acid scarification, boiling water, dry heat, soaking, and plant growth regulators) of *S. birrea* seeds did not promote germination. Seeds of *S. birrea* can be considered orthodox because they tolerated desiccation without significant loss of viability. Both intact and opercula-removed seeds readily imbibe water suggesting physiological rather than physical dormancy. The highest germination percentage was recorded under constant dark conditions at 25 °C for opercula-removed seeds exposed to an after-ripening period of 12 months. This study indicates that after-ripening, light conditions, and cold stratification are critical factors for germination of *S. birrea* seeds.

Sclerocarya birrea is native to the semi-arid deciduous savannas of sub-Saharan Africa (Muok et al., 2007) and is one of the most highly valued indigenous trees in southern Africa (Von Teichman and Robbertse, 1986). Besides a number of medicinal uses (Eloff, 2001), the importance of *S. birrea* is underpinned by its diverse characteristics such as high levels of vitamin C and protein, quality stable oil, and the novel flavor of its fruit. The vitamin C content of *S. birrea* fruit juice is approximately four to five times more than the levels found in the average orange juice (Jaenicke and Thiong'o, 2000; Mojeremane and Tshwenyane, 2004). Bio-

chemical analyses indicate that the kernels have higher protein and oil content than most of the popular nuts, including walnuts, hazelnuts, chestnuts, and almonds (Wynberg et al., 2003).

Humankind has benefitted from *S. birrea* as a source of nutrition for more than 10,000 years (Nwonwu, 2006). Apart from contributing to rural diets, the fruit is used to brew an alcoholic beverage with an annual gross value of ≈\$80 to \$100 U.S. per household (Emanuel et al., 2005; Shackleton et al., 2008). The oil is in high demand in the pharmaceutical and cosmetic industries (Kleiman et al., 2008; Nwonwu, 2006), whereas the nuts are used in the food industry for making a range of products, including chocolates. With the realization of its market value, there is notable growth in the trade of *S. birrea* products stimulated by local and industrial demand (Emanuel et al., 2005).

Owing to its economic potential, *S. birrea* has been earmarked for crop development and improvement in southern Africa (Molle-

and Goyvaerts, 2004). As industrial demand for its products is increasing, there is a growing concern about the sustainable supply (Nwonwu, 2006) and conservation of wild populations. Thus, in the quest for the domestication and improvement of *S. birrea*, understanding of seed germination of this plant is essential. Several members of Anacardiaceae, including *Sclerocarya*, are characterized by a drupe fruit with a stony endocarp. The endocarp of *Sclerocarya* and other related genera has a specialized structure, the operculum, through which the germinating embryo emerges (Von Teichman and Robbertse, 1986). Hills (1933) stated that the Anacardiaceae exhibits some remarkable seed protection mechanisms by means of a hard lignified endocarp and, intriguingly, the most ingenious devices to allow emergence of the germinating embryos. This ingenious opening device (operculum) represents one of the most sophisticated opening mechanisms in the germination of seeds (Von Teichman and Robbertse, 1986). Typical of the Anacardiaceae, the germinating unit (seed) in *S. birrea* is the true seed plus endocarp (Gaméné et al., 2004; Li et al., 1999).

Although considerable research has been undertaken on this species, there is still a dearth of knowledge on some aspects of its seed biology. Gaméné et al. (2004) inconclusively suggested that seed after-ripening, a decrease in mechanical resistance of the operculum after storage, or a combination of both factors can improve germination of *S. birrea* seeds. Another research gap relates to the effect of light on the germination process (Von Teichman et al., 1986). Generally, under natural conditions, temperature, light, water, oxygen, and mechanical pressures are some of the important factors that can influence seed germination of species like *S. birrea*. The purpose of this study was to identify the possible environmental and mechanical cues influencing the germination of *S. birrea* seeds.

Materials and Methods

Seed collection. Fruits of *Sclerocarya birrea* were collected in Feb. 2007 from the Mpumalanga Province of South Africa. Fruits were depulped and cleaned as described in the seed leaflet of the Danida Forest Seed Centre (2003). The seeds (endocarps) were separated from the pulp, washed, dried, and stored in brown paper bags at room temperature (22 ± 2 °C) for 4 weeks before being tested for germination ability. Seeds used to determine the initial moisture content were not stored.

Seed germination. Before each germination test, seeds were surface-decontaminated by soaking for 15 min in 0.5% (w/v) solution of mercuric chloride (HgCl₂). Subsequently, seeds were thoroughly rinsed under tap and then distilled water. Before the germination experiments, seeds were soaked for 24 h in the dark at room temperature (22 ± 2 °C) in distilled water (covering 75% of the seed) for

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the hard endocarps to imbibe water. Seed germination was carried out on cotton wool moistened with distilled water and placed in plastic containers (10.5 × 10.5 × 13.5 cm) in growth chambers equipped with cool white fluorescent lamps (Osram L 58W/640, München, Germany) emitting a photosynthetic photon flux density (*PPFD*) of ≈100 μmol·m⁻²·s⁻¹ over a wavelength band of 400 to 700 nm. Light intensity was measured with a quantum radiation sensor (Model Skp 215; Skye Instruments Ltd., Llandrindod Wells, Powys, UK). Seeds were considered to have germinated when the radicle had emerged at least 2 mm (Bewley, 1997). Each treatment consisted of 25 seeds and was replicated four times. All the experiments were repeated twice. Germination was recorded daily. The seeds that were subjected to continuous dark conditions were examined under a “green safe light” (wavelength of 510 nm and *PPFD* of 0.2 μmol·m⁻²·s⁻¹) in the dark (Kulkarni et al., 2006). Seeds that were not treated served as a control unless mentioned otherwise. Unless stated otherwise, the duration of germination experiments was 14 d.

Moisture content determination. True seeds (embryonic axis and cotyledons) (Fig. 1) were excised from the stony endocarp of marula seeds and moisture content was determined gravimetrically by weighing before and after oven-drying at 110 °C for 48 to 96 h until a constant weight was obtained. Moisture content was calculated on the basis of fresh weight: (% moisture content) = (fresh weight – dry weight)/fresh weight × 100. The results represent the means of the moisture content values of 20 embryos (± SE) obtained from two separate experiments.

Water uptake. Water uptake was determined using both intact (Fig. 1) and opercula-removed (Fig. 1) seeds that had been stored at ambient room temperature in the dark for 12 months. Intact and opercula-removed seeds (25 seeds per replicate) were placed on cotton wool moistened with distilled water in plastic containers (10.5 × 10.5 × 13.5 cm) and incubated under cool fluorescent white light (16-h light:8-h dark) with a *PPFD* of 60 μmol·m⁻²·s⁻¹ at room temperature (22 ± 2 °C). Seeds were blotted dry with a paper towel, weighed, and replaced in containers at

4-h intervals for 96 h. Percentage water uptake was calculated on the basis of actual increase in seed mass over the initial seed mass (Hidayati et al., 2001):

$$\%W_s = [(W_i - W_d)/W_d] \times 100$$

where W_s = increase in mass of seeds; W_i = mass of seeds after a given interval of imbibition; and W_d = initial mass of seeds.

Effect of temperature. Intact seeds were soaked in distilled water for 24 h before exposing them to different temperatures. Seed germination was determined for both intact and opercula-removed seeds under alternating light (16-h photoperiod of 100 μmol·m⁻²·s⁻¹) and continuous dark conditions (containers were wrapped with aluminum foil). The seeds were incubated at constant temperatures of 10, 15, 20, 25, 30, 35, and 40 °C and alternating temperature of 30/15 °C (14 h/10 h) in plant growth chambers (Controlled Environments Ltd., Manitoba, Canada). The optimum temperature for germination was determined on the basis of constant temperatures using the formula (Kulkarni et al., 2006):

$$T_o = \sum tp / \sum p$$

where T_o is the optimum temperature for germination and p is the percentage germination at temperature t .

Effect of seed after-ripening on germination. Intact seeds with a moisture content of 11.1 ± 1.6% (fresh weight basis) were stored in closed brown paper bags for 6, 9, and 12 months in the dark at room temperature (22 ± 2 °C) after which germination was evaluated. After 6, 9, and 12 months, seeds were removed from storage and tested for their germination response to temperature and light. For cold and warm stratification experiments, only seed stored for 12 months was tested for germination.

Effect of irradiance intensity. Opercula-removed seeds were soaked in distilled water for 24 h as described earlier. Seeds were then exposed to continuous *PPFD* of 0 (dark), 20, and 115 μmol·m⁻²·s⁻¹ under cool white fluorescent lamps and incubated at a temperature of 25 ± 2 °C.

To examine the effect of different light spectra, seeds were placed in boxes fitted with red (1.5 μmol·m⁻²·s⁻¹), far-red (1.4 μmol·m⁻²·s⁻¹), blue (0.2 μmol·m⁻²·s⁻¹), and green (0.2 μmol·m⁻²·s⁻¹) light filters and incubated under continuous light (100 μmol·m⁻²·s⁻¹) using cool white fluorescent lamps (Osram L 58W/640) at 25 ± 2 °C (Kulkarni et al., 2006). Incubation in the dark served as the control. Percentage germination was recorded after 7 d.

Pregermination treatments. Intact seeds were used for all the pregermination treatments. For all pregermination experiments, controls consisted of untreated seeds. Seeds were incubated on cotton wool moistened with distilled water. For the acid scarification treatment, seeds were soaked in 96% (v/v) sulfuric acid (H₂SO₄) for 2, 4, 6, 8, and 10 h.

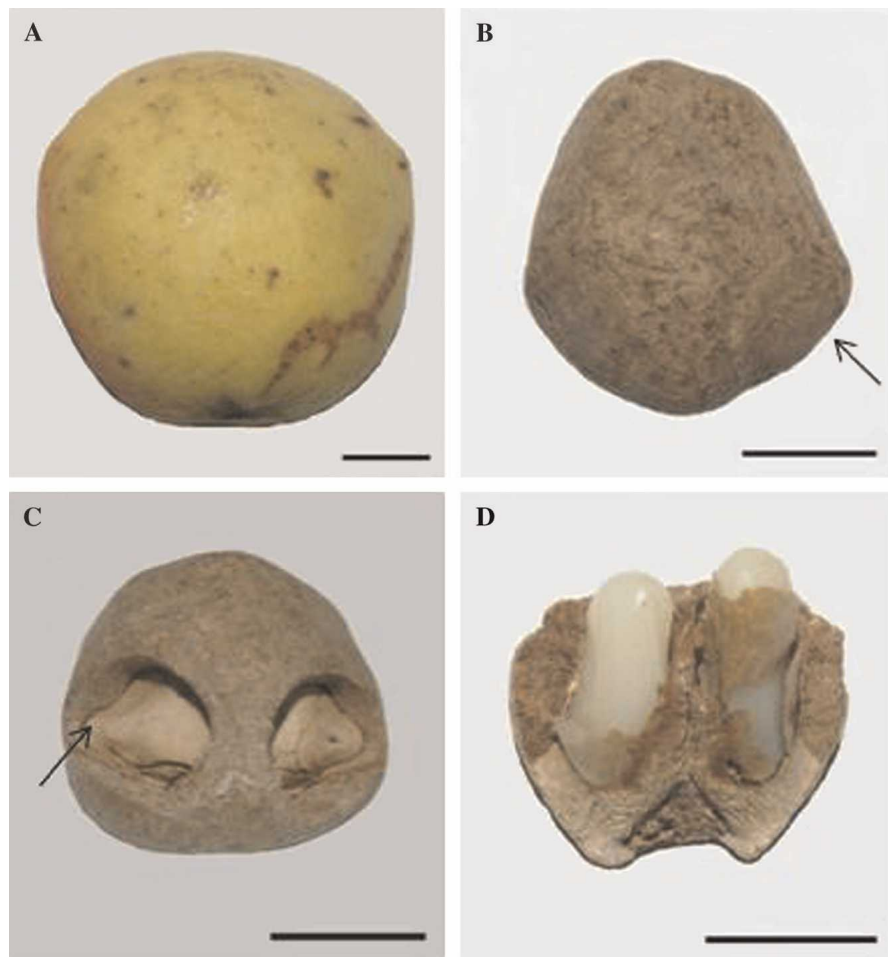


Fig. 1. Structure of a marula fruit, endocarp, and seed. (A) Ripe marula fruit. (B) An intact marula nut showing the hard lignified endocarp after removal of the fruit pulp. The weight of an intact marula endocarp after 12 months of storage at room temperature was 5.06 ± 0.45 g (n = 100). The arrow indicates the operculum, a potential physical barrier to germination, through which the radicle emerges. The weight of an operculum was 0.195 ± 0.025 g (n = 100). (C) An opercula-removed marula endocarp. The arrow indicates the position of the embryo. (D) Vertical section of a marula nut showing the walls of the stony endocarp and the true seeds. Scale bar = 10 mm.

Subsequently, the seeds were rinsed thoroughly in water for 30 min. In another treatment, the seeds were subjected to boiling water for 5, 10, 15, 20, 25, and 30 min; removed; and left to cool for 30 min. For the dry heat treatment, seeds were placed in the oven and exposed to 110 °C for 2, 4, 6, 8, and 10 h. For soaking treatments, seeds were placed in distilled water for 12, 24, 48, 72, 96, and 168 h at ambient room temperature in the dark. After each treatment, the seeds were soaked in distilled water for 24 h (excluding soaking treatments) and incubated in a growth chamber (Conviron; Controlled Environments Ltd.) under continuous dark conditions at 25 ± 2 °C. Kinetin, gibberellic acid, and potassium nitrate (KNO₃) were tested at 0.1, 0.01, and 0.001 μM concentrations under both continuous light and continuous dark conditions. Seeds were soaked in these solutions for 24 h and incubated on cotton wool moistened with distilled water at 25 ± 2 °C.

Seed stratification. Intact seeds were placed between two layers of paper towel, moistened with distilled water (using a 500-mL plastic spray bottle), and kept inside plastic bags. These bags were then stored in the dark at 5 °C (cold stratification) and 40 °C (warm stratification) for 7, 14, 21, and 28 d. For each treatment, four plastic bags were incubated at the respective temperature. After the respective incubation periods, germination tests were conducted under continuous dark conditions at 25 ± 2 °C. The seeds used for the stratification experiments were 12 months old.

Statistical analysis. Seed germination data were expressed as mean values ± SE. The germination percentage data were arcsine-transformed before statistical analysis to ensure homogeneity of variance. One-way analysis of variance was conducted and Tukey's test was used to separate differences among treatment means. Data were analyzed using SPSS Version 15 (SPSS®, Chicago, IL).

Results

Seed moisture content. *Sclerocarya birrea* seed stored for 12 months at room tempera-

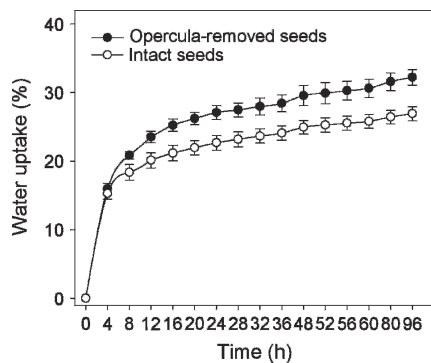


Fig. 2. Water uptake for *Sclerocarya birrea* subsp. *caffra* seeds during 96 h of incubation under alternating light (16-h photoperiod) at a room temperature of 22 ± 2 °C (n = 25).

ture had a mean fresh weight of 5.06 ± 0.45 mg (n = 100). The moisture percentage of excised true seeds from fresh nuts was 11.1 ± 1.6%, which was significantly higher than that of 12-month-old nuts (4.9 ± 0.57%). The reduction in seed water content was achieved after 48 h at 110 °C.

Imbibition. Water uptake by both 12-month-old intact (26.9 ± 1.03%) and opercula-removed (32.2 ± 1.16%) seeds followed a similar imbibition curve, although water uptake for intact seeds was slightly lower (Fig. 2). Initially, in both intact and opercula-removed *S. birrea* seeds, the rate of water uptake up to 8 h was rapid and slowed down thereafter.

Effect of temperature and light on germination. At all the temperatures tested, alternating light (16-h photoperiod) was inhibitory for seed germination of 9-month-old opercula-removed seeds. Opercula-removed seeds exhibited higher percentage germination than intact seeds between 20 and 35 °C under constant dark conditions (Table 1). Low temperatures (10 and 15 °C) inhibited seed germination of both intact and opercula-removed fruits. At high constant temperature regimes (25 to 40 °C), 12-month-old intact seeds resulted in better germination than 9-month-old intact seeds. A similar trend was noted for alternating temperature (30/15 °C) (Table 1).

After 6 months of storage, percentage germination of opercula-removed seeds was significantly higher under continuous dark in comparison with both continuous and alternating light conditions in which no germination was recorded (Fig. 3A). However, the inhibitory effect of white light was significantly reduced after 12 months of seed storage, exhibiting 65.2 ± 0.9% and 67.2 ± 1.3% germination under continuous and alternating light conditions, respectively (Fig. 3B). Intact seeds stored for up to 9 months did not germinate (data not shown), but after 12 months of storage, the seeds germinated equally in continuous dark (31.3 ± 4.4%), continuous light (32.7 ± 2.6%), and alternating light (30.5 ± 3.8%) conditions (Fig. 3C). For the same storage period, percentage germination of opercula-removed seeds showed no significant difference at PPF_D ranging from 0 to 115 μmol·m⁻²·s⁻¹ (Table 2).

Opercula-removed seeds that did not germinate under a 16-h photoperiod at a temperature of 25 °C were moved to continuous dark conditions at 25 °C resulting in the reversal of the photoinhibition effect (Fig. 4). Similarly, 9-month-old seeds that did not germinate at 10, 15, and 20 °C showed significantly high germination percentages when shifted to 25 °C in the dark (Fig. 4).

Red and blue light spectra had a stimulatory effect on the germination of 6-month-old opercula-removed seeds (Table 3). However, the sensitivity of opercula-removed seeds to different light spectra was reduced after prolonged seed storage of 12 months (Table 3).

Effect of after-ripening on germination. Intact seeds of *S. birrea* germinated to 33.2 ± 5.9% at 30 °C under constant dark conditions after a 12-month after-ripening period (Table 1). White light inhibited germination of 6-month-old opercula-removed seeds under both continuous and alternating light conditions. However, dark conditions promoted germination of *S. birrea* irrespective of the after-ripening period of the seeds (Figs. 3A–B). After 12 months of seed storage, the photoinhibition effect was partially lost with significant improvement in final germination (Fig. 3B).

Effect of pregermination treatments. All pregermination experiments were conducted on 12-month-old seed. Scarification with sulfuric acid (H₂SO₄) did not improve germination of *S. birrea* seed relative to the control (data not shown). Boiling water and dry heat also did not improve seed germination of *S. birrea* in comparison with the controls. The germination for dry heat and boiling water treatments was 0% indicating that the seeds probably were killed. Furthermore, neither prolonged soaking (*P* > 0.420) nor application of plant growth regulators (*P* > 0.665) and KNO₃ (*P* > 0.882) were effective in enhancing germination of *S. birrea* seeds.

Cold stratification. The seeds of *S. birrea* subjected to cold stratification for a period of 14 d at 5 °C showed significantly greater germination (65%) compared with nonstratified (32%) and seeds that were cold-stratified for 7, 21, and 28 d (less than 32%) (Fig. 5). Warm stratification did not improve seed germination (*P* > 0.348).

Table 1. Effect of different temperatures on *Sclerocarya birrea* susp. *caffra* seed germination under constant dark conditions at 25 ± 2 °C.

Temperature (°C)	Germination (%)			
	9-month-old seeds		12-month-old seeds	
	Opercula-removed	Intact	Opercula-removed	Intact
10	0 ± 0 c	0 ± 0 a	0 ± 0 c	0 ± 0 c
15	0 ± 0 c	0 ± 0 a	0 ± 0 c	0 ± 0 c
20	52.6 ± 6.8 b	0 ± 0 a	54.5 ± 4.3 b	6.2 ± 4.0 bc
25	91.3 ± 1.5 a	0 ± 0 a	94.8 ± 1.2 a	20.8 ± 5.3 ab
30	87.0 ± 2.9 a	0 ± 0 a	90.6 ± 1.8 a	33.2 ± 5.9 a
35	87.0 ± 6.4 a	0 ± 0 a	86.2 ± 5.8 a	20.8 ± 7.9 ab
40	25.0 ± 5.9 bc	0 ± 0 a	26.2 ± 6.1 bc	31.2 ± 8.5 ab
30/15	86.4 ± 5.1 a	0 ± 0 a	92.4 ± 4.2 a	18.0 ± 3.4 ab

Values (± SE) with different letters in a column are significantly different at 5% level of significance according to Tukey's test (*P* < 0.05).

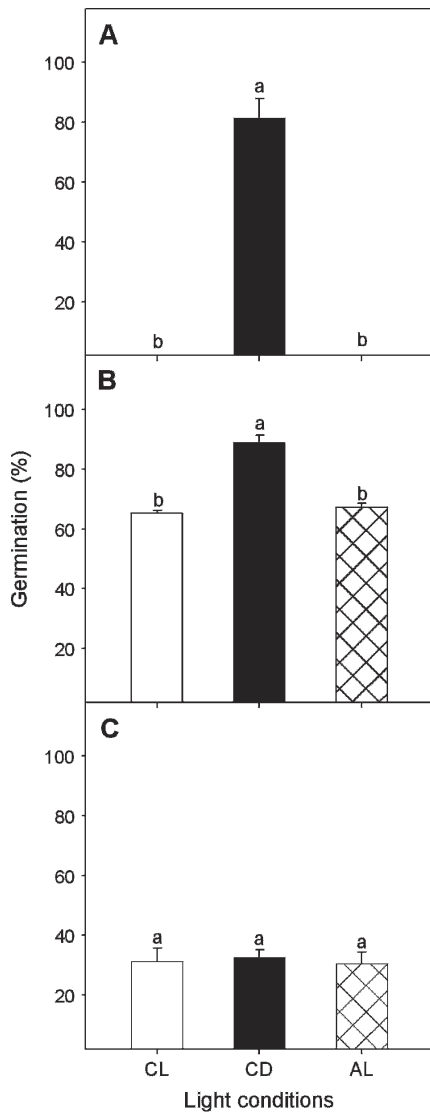


Fig. 3. Effect of different light conditions and seed storage on percentage germination of *Sclerocarya birrea* subsp. *caffra* seeds incubated at 25 ± 2 °C. CL = continuous light; CD = continuous dark; and AL = alternating light (16-h photoperiod). Seeds were soaked in distilled water for 24 h before germination tests. (A) Germination of opercula-removed seeds after a 6-month storage period. (B) Germination of 12-month-old opercula-removed seeds. (C) Germination of intact seeds after a 12-month storage period. The opercula were removed just before the germination tests. Six-month-old intact seeds did not germinate at all light conditions tested. Bars (\pm SE) with similar letters are not significantly different at 5% level of significance according to Tukey's test ($P < 0.05$).

Discussion

Seed storage under dark conditions at room temperature for 12 months resulted in moisture loss from the seed. The difference in seed moisture content between fresh and 12-month-old seeds could explain the higher germination percentage after the prolonged storage period. On the basis of its tolerance to low moisture content of $4.9 \pm 0.57\%$, *S*

Table 2. Effect of irradiance intensity on percentage germination of *Sclerocarya birrea* subsp. *caffra* seeds at 25 ± 2 °C.

Storage period (months)	Light intensity ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)		
	0	20	115
6	80.9 ± 5.3 b	0 ± 0 b	0 ± 0 b
9	91.3 ± 1.5 a	0 ± 0 b	0 ± 0 b
12	91.7 ± 1.0 a	86.9 ± 2.7 a	90.4 ± 3.7 a

Values (\pm SE) with different letters in a column are significantly different at 5% level of significance according to Tukey's test ($P < 0.05$).

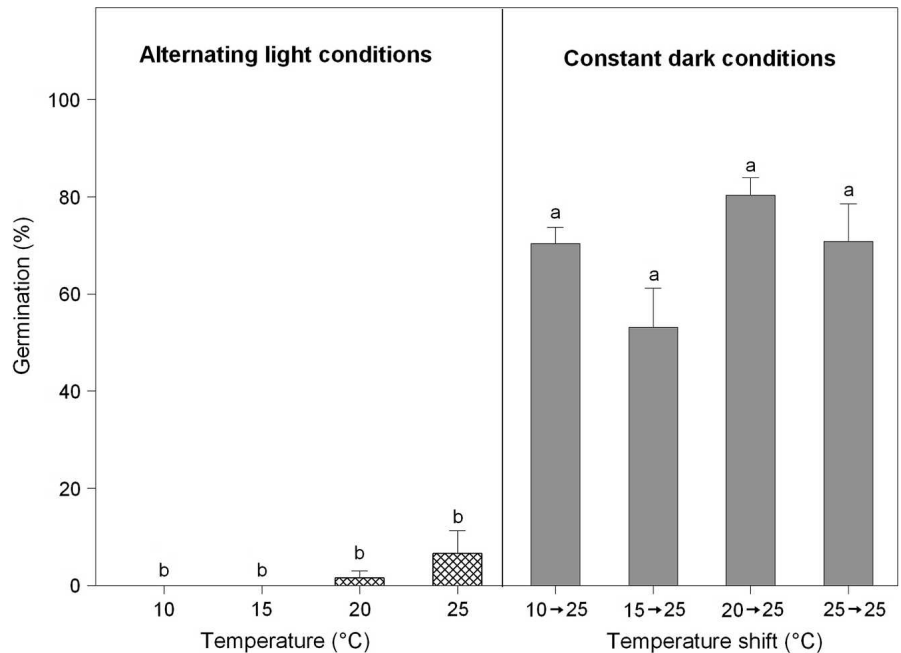


Fig. 4. Effect of temperature and light shifts on percentage germination of *Sclerocarya birrea* subsp. *caffra* seeds after a storage period of 9 months. The seeds that had been after-ripened for 9 months were shifted from alternating light to constant dark conditions after 14 d. The graph shows that the inhibitory light effect is reversible. Bars (\pm SE) with similar letters are not significantly different at 5% level of significance according to Tukey's test ($P < 0.05$).

birrea seeds can be classified as orthodox. This confirms the findings of Pritchard et al. (2004). According to Pritchard et al. (2004) and Tweddle et al. (2003), desiccation-tolerant or orthodox seeds can tolerate low moisture content below 7% and subsequently rehydrate without significant variation in viability. Generally, trees and shrubs adapted to arid and highly seasonal environments are overwhelmingly desiccation-tolerant (Tweddle et al., 2003).

The large seed of *S. birrea* exhibited typical characteristics of species found in dry tropical regions, where seeds have relatively large amounts of nutrients, to support rapid growth of seedlings, thereby increasing their chances of survival (Tweddle et al., 2003). Both intact and opercula-removed *S. birrea* endocarps readily imbibe water (Fig. 2). Similarly, intact *Lanea microcarpa* (Anacardiaceae) seeds imbibed water, although the rate of water uptake was faster for scarified endocarps (Neya et al., 2008). In contrast, the seeds of other species of Anacardiaceae such as *Rhus aromatica* and *R. glabra* were not permeable to water, even when their outer two layers (brachysclereids

Table 3. Effect of different light spectra on *Sclerocarya birrea* subsp. *caffra* seed germination at 25 ± 2 °C.^z

Light source	Germination (%)	
	6-month-old seeds	12-month-old seeds
White light	0 d	79.1 ± 4.1 a
Dark	81.3 ± 6.5 a	83.3 ± 0.0 a
Red	53.2 ± 7.5 ab	80.5 ± 8.6 a
Far-red	32.8 ± 12.1 bc	61.1 ± 6.3 b
Green	24.7 ± 5.1 c	61.1 ± 2.4 b
Blue	46.6 ± 17.8 b	77.7 ± 2.4 a

^zOpercula-removed seeds were soaked in distilled water for 24 h before germination test. Values (\pm SE) with different letters in a column are significantly different at 5% level of significance according to Tukey's test ($P < 0.05$).

and osteosclereids) were removed (Li et al., 1999).

Because *S. birrea* is adapted to the drier tropical regions, high temperatures in the range of 25 to 35 °C favors seed germination. In this study, the calculated optimum temperature (T_o) for the germination of *S. birrea* seeds was 29 °C under constant dark conditions. Figure 4 shows the effect of both light

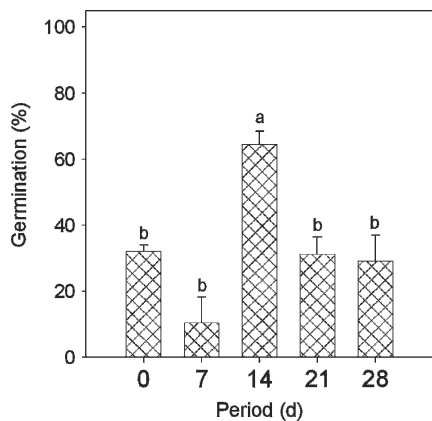


Fig. 5. Effect of cold stratification (5 °C) on germination of intact *Sclerocarya birrea* subsp. *caffra* under continuous dark conditions at 25 ± 2 °C. The seeds were cold-stratified after a 12-month storage period. Bars (± SE) with similar letters are not significantly different at 5% level of significance according to Tukey's test ($P < 0.05$).

and temperature shifts for *S. birrea* seeds after 9 months storage. When opercula-removed seeds did not germinate at 10 and 15 °C or showed little germination at 20 and 25 °C under a 16-h photoperiod, they were shifted to continuous dark at 25 °C, which significantly increased percentage germination. Continuous exposure of seeds to specific light spectra had a significant influence on percentage germination of 6-month-old seeds of *S. birrea* (Table 3). In particular, red and blue light increased seed germination as compared with white light for the 6-month-old seed. These results may indicate the influence of phytochrome family of photoreceptors on seed germination. The inhibitory effect of white light may be the result of a high rate of interconversion between the P_{fr} and P_r forms of phytochrome caused by high PPF of light of any wavelength (Ellis et al., 1989). This high irradiance reaction overrides the reversible phytochrome reactions and may be inhibitory to seed germination (Baskin and Baskin, 1998). The stimulatory influence of both red and blue light observed for 6-month-old seed was lost with prolonged storage of seeds (12 months) at room temperature (Table 3).

Another factor influencing seed germination is after-ripening in storage. Von Teichman et al. (1986) and Gaméné et al. (2004) reported that final germination of *S. birrea* seeds increased after 1 year and 6 months of storage under ambient temperature and relative humidity conditions, respectively. This shows that after-ripening of seeds during storage at ambient temperatures is critical for germination of *S. birrea*. Using opercula-removed seeds, Pritchard et al. (2004) showed that physiological rather than physical dormancy had a predominant influence on seed germination of *S. birrea*. For *L. microcarpa*, the increase in germination with drying was attributed to seed after-ripening and/or a loss of physiological dormancy (Neya et al., 2008). It is further suggested

that desiccation tolerance is greatest for seeds exhibiting physical and combinational (physical and physiological) dormancy (Tweddle et al., 2003).

Intact seeds of *S. birrea* stored up to 6 months failed to germinate under all the light conditions (continuous light, continuous dark, and alternating light) examined. However, after 12 months of storage, there was a partial loss of dormancy (Fig. 3C). Similarly, for *Prosopis juliflora*, storage of seeds significantly achieved greater and faster germination (El-Keblawy and Al-Rawai, 2006). The germination response of *S. birrea* seeds to light and storage suggests both physiological and endocarp-imposed dormancy.

Von Teichman et al. (1986) showed that acid scarification was not effective in enhancing seed germination of *S. birrea*. However, Gaméné et al. (2004) reported an increase in germination after treating the seeds with hydrochloric acid (HCl). Li et al. (1999) have reported that concentrated H_2SO_4 released seed dormancy of *R. aromatica*, and boiling water that of *R. glabra*, both members of Anacardiaceae. For *S. birrea* scarification with H_2SO_4 , boiling water, dry heat, and prolonged soaking of seeds did not improve germination.

Seeds of some species with stony endocarps germinate better when they are subjected to different periods of cold stratification, e.g., *Cornus* (90 to 120 d), *Coryllus* (60 to 180 d), *Menispermum* (14 to 28 d), *Morus* (30 to 90 d), *Nyssa* (30 to 120 d), and *Oemleria* (120 d) (Young and Young, 1992). For *S. birrea* seeds, a cold stratification treatment of 14 d significantly increased germination (Fig. 5). The response to cold stratification indicates adaptive mechanisms and significance of natural environmental cues such as low winter temperatures to which *S. birrea* seeds may get exposed before germination.

Conclusions

This study has identified the factors that influence the germination of *S. birrea*. Both intact and opercula-removed seeds readily imbibe water suggesting physiological rather than physical dormancy. Light had an inhibitory effect on opercula-removed seeds, which was subsequently eliminated after prolonged storage at ambient temperature. Seeds of *S. birrea* can be considered orthodox because they tolerated desiccation. The highest germination was recorded under constant dark conditions at 25 °C for opercula-removed seeds exposed to an after-ripening period of 12 months. The findings of this study indicate that after-ripening, light, temperature, and cold stratification are critical determinants for the germination of *S. birrea* seeds.

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