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Seed dormancy, germination and seedling characteristics of *Elaeocarpus prunifolius* Wall. ex Müll. Berol.: a threatened tree species of north-eastern India

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Abstract

Background: *Elaeocarpus prunifolius* Wall. ex Müll. Berol. is a threatened tree species of north-eastern India. The present study was undertaken to investigate the type of dormancy prevailing in seeds of *E. prunifolius*, explore seed dormancy breaking techniques and assess seedling fitness.

Methods: Ripe fruits of *E. prunifolius* were harvested from Jaintia hills, and seeds were subjected to various physical, manual and chemical treatments. The effect of plant growth regulators, viz gibberellic acid (GA_3) and potassium nitrate (KNO_3), were tested. Seedling vigour and survival based on seed weight were examined.

Results: Germination took 6 months to initiate after seed dispersal and natural germination percentage of fresh seeds was 24%. Physical pre-germination treatments such as surface and acid scarification failed to overcome dormancy. Cracked seeds promoted germination (46%) with a mean germination time of 146 days (time to 50% germination, $T_{50} = 144$ days). Among the GA_3 treatments, split seeds treated with GA_3 (3000 mg/L^{-1}) yielded the highest germination (24%) with a T_{50} of 55 days whereas KNO_3 did not promote germination. A combination of GA_3 and KNO_3, however, increased the germination to 31%. Between the seed weight classes, the highest percentage of germination was observed in heavy seeds (25%) and the lowest in light seeds (20%). There was no significant variation between seed weight and germination time ($p > 0.05$). Seed weight had a significant effect on the shoot height, number of leaves and dry weight of seedlings ($p < 0.05$).

Conclusion: Based on the seed tests, *E. prunifolius* seeds exhibits 'combined' dormancy (physical and physiological) as splitting seed coat and application of GA_3 effectively broke dormancy. Splitting the seed coat is a cost-effective method for accelerating germination of seeds. Heavy-weight seeds produced better performing seedlings compared to their counterparts which may be viewed as an important reproductive strategy of the species.

Keywords: Conservation, *Elaeocarpus*, Germination, Seedling growth, Threatened

Background

In recent years, studies on germination have emerged as an important tool for conservation of many species. Such studies aid understanding of natural regeneration processes as well as identifying possible causes of species decline, persistence or spread in changing landscapes and their response to global climate change (Schütz 2000; Gill et al. 2013; Tanaka-Oda et al. 2017). Thus, such studies enable the development of suitable protocols for species conservation and management.

The genus *Elaeocarpus* have ca. 360 species distributed across East Asia, Australia, Malaysia and the Pacific Islands. The International Union for Conservation of Nature and Natural Resources (IUCN) have identified 38 *Elaeocarpus* species under various threat categories. Of these, 3 species are critically endangered, 4 endangered, 20 vulnerable, 2 near threatened, 8 conservation dependent and 1 data deficient (IUCN 2017). In India,

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30 species occur (Murti 1993a, b), the majority of which are confined to the northeast (Assam, Arunachal Pradesh, Sikkim, Manipur, Meghalaya, Mizoram, Nagaland, Tripura) and south (Tamil Nadu and Kerela) India (Khan et al. 2003; Singh et al. 2013). Out of the six threatened *Elaeocarpus* species reported from the country, one species (*E. gaussenii* Weibel) is critically endangered and one endangered (*E. blascoi* Weibel), three are Vulnerable (viz. *E. prunifolius* Wall. ex Müll. Berol, *E. recurvatus* Corner and *E. venustus* Bedd.) and one (*E. munroii* Mast.) falls under lower risk/near threatened category. Of these, *E. prunifolius* is restricted to north-eastern India and the others are confined to southern India.

Research on *Elaeocarpus* species has gained momentum with the discovery of indolizidine alkaloid compounds in members such as *E. fuscoides* Knuth., *E. grandis* F. Muell., *E. polydactylus* Schltr. and *E. densiflorus* Knuth (Katavic 2005) for their potential treatment of diseases such as AIDS, diabetes and cancer (Wiart 2006). In addition, fruits of species, viz *E. floribundus* Blume, *E. lanceifolius* Roxb., *E. braceanus* Watt ex C.B. Clarke and *E. sikkimensis* Mast, are edible (Andola et al. 2011; Das 2014) and species such as *E. grandis*, *E. ruminatus* F.Muell., *E. foviolatus* F. Muell. and *E. coorangooloo* J.F. Bailey & C.T. White are known for their good timber value (Bristow et al. 2005).

Elaeocarpus prunifolius is a middle-sized tree that attains a height of ca. 20 m at maturity (Nayar and Sastry 1990). The phenological calendar of the species is presented in Table 1. The inflorescences are 3–9 cm long, and the flowers are pale yellow in colour. The ovoid fruits range from 0.8 to 1.2 cm in diameter. They change from green to bluish-black on maturation. There is only one seed per endocarp. The ripe fleshy mesocarp is predated by worms, insects and birds, and seeds are predated by small rodents, thus, dispersing the seeds in the process (epizoochory) (field observation).

Elaeocarpus prunifolius is a rare and threatened tree species distributed in the state of Manipur and Meghalaya in India and Bangladesh (Murti 1993a, b; World Conservation Monitoring Centre 1998). In Meghalaya, the species is restricted to fragmented pockets of sub-tropical broad-leaved forests with only a few matured individuals (Walter and Gillett 1998; Nayar and Sastry 1990). The species is on the verge of extinction due to habitat degradation (Jain and Rao 1983; Haridasan and Rao 1985), which is further exacerbated by its on-going exploitation for timber by local communities (Nayar and Sastry 1990). The fruits of the species are also edible.

Propagation of *Elaeocarpus* species from seed is challenging, as the seeds exhibit dormancy (Khan et al. 2003). The fruits of *E. prunifolius* mature in early September and remain dormant throughout the winter (November–February). The seeds germinate in April with the onset of rain so moisture may be a requirement for germination. The inherent stony endocarp in many members of the genus seems to be a plausible factor for poor germination (Bhuyan et al. 2002; Khan et al. 2003; Ramasubbu and Irudhyaraj 2016). Some studies have revealed low (1–3%) and erratic seed germination in *Elaeocarpus ganitrus* Roxb. ex G.Don (Khan et al. 2003) and *E. japonicus* Siebold (Yang et al. 2001). Natural regeneration of the threatened *E. blascoi* was only 5% in the wild (Ramasubbu and Irudhyaraj 2016) while in *E. venustus*, *E. serratus* L and *E. williamsianus* Guymer, there was no germination (Saravanan et al. 2011; Dahanayake et al. 2013; Rossetto et al. 2004). Contrastingly, some species such as *E. floribundus* had fairly high germination (47%) (Das 2014). However, there is a dearth of knowledge (particularly on germination) of threatened species often targeted for conservation (Baskin and Baskin 2001). Khan et al. (2003) studied the germination capacity of *E. ganitrus* by subjecting the seeds to various treatments and found that seeds cracked with a vice resulted in highest germination percentage (40%) followed by seeds soaked in hot water for 24 h and seeds fermented for 20 days (37%). A mixture of silt and sand media resulted in 40% germination in *E. venustus* whereas seeds sown in a media of sand, silt and cow dung germinated to about 8% (Irwin et al. 2013). In *E. serratus*, seeds treated with 50% HNO_3 germinated to 15% (Dahanayake et al. 2013). However, there is lack of information on the regeneration capacity of *E. prunifolius*. The poor regeneration of the species in nature could be due to

Table 1 Phenological calendar of *E. prunifolius*

Phenological events	Months											
	J	F	M	A	M	J	J	A	S	O	N	D
LF	■	■										
LM			■	■	■							
LFA									■	■		
FL		■	■									
FR					■	■	■	■	■			
FM									■			

LF leaf flushing, *LM* leaf maturation, *LFA* leaf fall, *FL* flowering, *FR* fruiting, *FM* fruit maturation

hard seed coat and/or prolonged dormancy period. The results presented here form part of a larger study to assess the causes of the species rarity in nature by understanding the species phenology, mode of dispersion, predation, seed physiology, germination pattern, seedling characteristics and factors debilitating seedling establishment. The objectives of the current work were to: (i) understand the type of dormancy present in the seeds of *E. prunifolius* by subjecting the seeds to a set of physical and chemical treatments; (ii) test if total germination and germination rate were influenced by seed weight; and (iii) examine if the seedling growth and survival were influenced by seed weight. The results are discussed in relation to possible factors responsible for low recruitment of this species in nature.

Materials and methods

Seed source

Ripe fruits of *E. prunifolius* were harvested during mid-September 2013 from Jarain (25° 19.05′ N, 92° 08.34′ E, alt 1200 m asl), the known location of the species in Meghalaya, Northeast India (Haridasan and Rao 1985). As the species is rare, all mature trees were marked wherever encountered and monitored for fruit maturation. Fruits were collected randomly from ten randomly selected trees and bulked together to form a composite sample (ca. 4500 fruits). The fruits were brought to the laboratory and soaked in normal tap water (18 °C ± 1 °C for 24 h) to soften the pulp and also to separate healthy seeds from damaged ones (Pipinis et al. 2011). Empty or damaged seeds are lighter and float to the surface and were thus separated from healthy seeds. The fruits were hand crushed and the seeds collected. The seeds were washed thoroughly in running water to remove any adhering substance from the endocarp and disinfected in 0.2% potassium permanganate ($KMnO_4$) solution for 2 h following the method of Zuo (1994). The seeds were placed on paper to remove excess moisture and were subjected to various dormancy-breaking treatments within 5 days of seed collection. The remaining seeds were stored in an airtight container filled with a substrate (moist sand) and stored at a constant temperature of 5 °C (±1 °C) in a refrigerator for further studies.

Water imbibition test

Twenty-five seeds were cracked with a vice, and the initial weight of each seed was measured. The weights of 25 uncracked seeds were also determined. Both sets of seed were then placed on Petri dishes (90 mm × 20 mm) lined with moist cotton and kept covered under identical laboratory conditions. Each seed was reweighed after 2, 4, 6, 8, 10, 20, 30 and 40 h, and the percentage change in mass was averaged from the 25 seeds from each set.

Seed treatments

(a) Seed coat impermeability is often associated with the presence of impermeable palisade layers of lignified cells (Vazquez-Yanes and Perez-Garcia 1976). Therefore, the following physical or chemical treatments were applied in an attempt to disrupt the seed coat. For each experiment, three replicates of 50 seeds (irrespective of weight) were maintained. Seeds were:
   1a. Soaked in cool water (20 °C) for 24 h
   2a. Soaked in hot water (80 °C) for 24 h till the water cooled
   3a. Soaked in boiling water (5 min in 100 °C and immediately transferred to cold water) for 24 h
   4a. Scarified by rubbing near the micropyle with sandpaper
   5a. Cracked lengthwise with a vice
   6a. Scarified near the micropyle using sandpaper and soaked in warm water (45 °C) for 2 h
   7a. Soaked in 95% H_2SO_4 for 5 min
   8a. Soaked in 95% H_2SO_4 for 10 min
   9a. Soaked in 95% H_2SO_4 for 15 min
   10a. Soaked in 95% H_2SO_4 for 20 min
   11a. A control was maintained by sowing seeds (also irrespective of seed weight) without any of the above-mentioned treatments.

The treated seeds were transferred to plastic trays filled with a mixture of garden soil and sand in the ratio of 3:1. Each tray was labelled and kept under laboratory conditions having an average temperature of 24 (± 1 °C) and light (> 700 lx) duration of 8 h. The trays were watered at 3-day intervals and monitored for germination for a period of 12 months.

(b) The effect of plant growth regulators (PGRs) on breaking seed dormancy and the rate of germination was examined by performing the following tests on seeds cracked lengthwise. Three replicates of 25 seeds were maintained for each treatment. Cracked seeds were:
   1b. Soaked in 200 mg L^{-1} gibberellic acid (GA_3) for 48 h
   2b. Soaked in 500 mg L^{-1} gibberellic acid (GA_3) for 48 h
   3b. Soaked in 1000 mg L^{-1} gibberellic acid (GA_3) for 48 h
   4b. Soaked in 2000 mg L^{-1} gibberellic acid (GA_3) for 48 h
   5b. Soaked in 3000 mg L^{-1} gibberellic acid (GA_3) for 48 h
   6b. Soaked in 0.5% potassium nitrate (KNO_3) for 48 h

7b. Soaked in 1% KNO_3 for 48 h
8b. Soaked in 1.5% KNO_3 for 48 h
9b. Soaked in 2% KNO_3 for 48 h
10b. Soaked in GA_3 solution (200 mg L^{-1}) for 48 h and transferred to 0.5% KNO_3 solution for another 48 h
11b. Soaked in GA_3 solution (500 mg L^{-1}) for 48 h and transferred to 1% KNO_3 solution for another 48 h
12b. Soaked in distilled water for 48 h

The control and treated seeds were placed in Petri dishes (90 mm × 20 mm) lined with moist filter paper and transferred to growth chambers fitted with an LED light source (> 4000 lx) with a photoperiod of 8 h. A constant temperature of 25 °C (± 1 °C) was maintained for germination. The germination of the seeds in the growth chambers was monitored at 3 d intervals for a period of 4 months.

Seed viability

One hundred and fifty untreated seeds were randomly selected from those stored at 5 °C for 12 months. Each seed was cracked lengthwise and the embryo excised. Each embryo was tested for viability following the tetrazolium assay of Wang et al. (2005). The presence of living cells in the seeds converts the TTZ (2,3,5 triphenyl tetrazolium chloride) to formazan (red colour) through the hydrogen transfer reaction catalysed by the cellular dehydrogenase, thus, staining the viable seeds red.

Seed germination and seedling growth

A subsample of 1500 seeds was used to examine the effect of seed weight on germination and seedling growth. Each seed was examined by the floatation method and 60 seeds were found to be damaged. The remaining 1440 healthy seeds were weighed individually. The number of seeds in each of three weight categories (light (< 320 mg), intermediate (320–480 mg) and heavy (> 480 mg)) was determined. Seeds from each category were sown under laboratory conditions in plastic trays (26 cm diameter, 8 cm height) within 10 days from the time of seed collection. A mixture of garden soil and sand media in the ratio of 3:1 was used for planting. Twenty-five seeds were sown per tray. Germination was recorded at 3-day intervals up to 6 months and weekly up to 12 months. The number of germinated seeds was recorded on each occasion. Seeds were considered germinated when the radicles protruded out of the soil surface.

Germinated seedlings (about 2 months old) were transplanted into 20 × 17 cm poly bags and transferred to the net house during May 2014. The moisture level was maintained by watering every 3 days. Seedling survival and growth were monitored for a period of 12 months after transplantation. To assess the effect of light on seedling growth, a threefold contrast in light level was created by covering the net houses with an increasing layer of shade netting. Each extra layer intercepted an additional 25% (approximately) of the incoming radiation, thus, creating three light regimes of approximately 70–75% (16 ± 1.8 mol m^{-2} day^{-1}), 25–30% (4 ± 0.46 mol m^{-2} day^{-1}) and 5–10% (1 ± 0.05 mol m^{-2} day^{-1}) designated as high, intermediate and low light treatments respectively. Light intensity was measured with digital lux meter (Lutron LX-101A) at three different times during the day (9.00 a.m., 12 noon and 3 p.m.) each month and the seasonal means were calculated.

To assess the effect of seed weight and light on growth performance of the seedlings, ten randomly selected seedlings under each weight category and light condition were harvested after one year of transplantation (May 2015) and their shoot height, root length, the number of leaves, leaf area and dry matter yield were determined. The leaf area was measured using a leaf area meter (LICOR, Lincoln). Dry matter yield was determined by drying the plant material in an oven at 80 °C for 24 h to a constant weight.

Germination percentage was calculated using the formula:

$$G(\%) = \frac{n}{N} \times 100,$$

where n is the number of germinated seeds and N is the total number of seeds. The method of Coolbear et al. (1984) further modified by Farooq et al. (2006) was used to calculate the time to reach 50% germination (T_{50}):

$$T_{50} = t_1 + \left[\left(\frac{N}{2} - n_i\right)\left(t_j - t_i\right)\right] / n_j - n_i,$$

where N is the final number of germinated seeds, n_i and n_j are the cumulative numbers of seeds germinated at times t_i and t_j, respectively, when $n_i < N/2 < n_j$.

Seed coat thickness

Seed-coat thickness was determined in each seed weight category to test the assumption that: (i) heavier seeds had thicker coats; and (ii) thicker seed coats may delay germination. Seeds were cut across and seed coat thickness was measured using a calliper and the values averaged. For each weight category, 30 seeds each were considered.

Data analysis

To determine the effect of different treatments on germination, analysis of variance (ANOVA) was used followed by Tukey and Scheffe's least significant difference ($p < 0.05$). Assumptions of ANOVA were met

through a test for normality of variables (Shapiro-Wilk test) and homogeneity of group variances (Levene's test). The relation between seed weight and seedling growth was analysed by regression. All the statistical analysis was performed using SPSS software (version 20).

Results

Imbibition test

The initial moisture content of scarified seeds was 10.6% and non-scarified seeds was 10.8%. After 40 h, both sets of seeds had a moisture content of 11.2% so the increase in the mass of scarified and non-scarified seeds was 6% and 4% respectively. This difference was not significant ($p > 0.05$) so uncracked seeds imbibe water at a similar rate as cracked seeds.

Dormancy-breaking treatments

Physical and chemical treatments 1a - 10a were compared to germination of untreated seeds (11a).

The germination percentage of control seeds was 24%. The mean number of days required for germination of control seeds was 213 ± 5 days (T_{50} = 174 ± 9 days). No germination of seeds occurred after 230 days. Only treatments 1a, 5a and 6a resulted in any germinated seedlings. Of these, treatment 1a (cold-water soak) reduced germination to 10.67% (T_{50} = 210 days) compared with the control (24%) while 14.67% of seeds (T_{50} = 216 days) germinated following treatment with a combination of scarification and warm water treatment (Treatment 6a). The germination percentage of seeds from these two treatments was significantly lower ($p < 0.05$) than the control. In contrast, 45.83% of seeds cracked with a vice germinated and this was significantly higher ($p < 0.05$) than for all other treatments. The mean germination time and T_{50} was also significantly reduced under this treatment (146 and 144 days respectively) (Table 2).

Treatments (1b-11b) involving soaking cracked seeds in solutions of various plant growth regulators were compared with germination of cracked seeds soaked in water (Treatment 12b), Table 2. Cracking seeds (Treatment 5a) resulted in better germination than no treatment (11a) but soaking seeds in water (Treatment 12b)

Table 2 Germination of *E. prunifolius* seeds subjected to (a) physical, mechanical and chemical scarification and (b) plant growth regulators

Treatment No.	Treatment details	Mean germination (days)	Germination (%)	T_{50} (days)
1a	Cold water (20 °C)	210 ± 1^{a}	10.67 ± 1.33^{a}	210 ± 2
2a	Hot water (80 °C)	0	0	0
3a	Boiling water (100 °C)	0	0	0
4a	Scarification near micropyle	0	0	0
5a	Cracked seeds with vice	146 ± 1	45.83 ± 1	144 ± 2
6a	Scarified+warm water (2 h)	212 ± 1^{a}	14.67 ± 1.33^{a}	216 ± 2
7a	95% H_2SO_4 for 5 min	0	0	0
8a	95% H_2SO_4 for 10 min	0	0	0
9a	95% H_2SO_4 for 15 min	0	0	0
10a	95% H_2SO_4 for 20 min	0	0	0
11a	Untreated control	213 ± 5^{a}	24.67 ± 3	174 ± 9
1b	200 mg L^{-1} GA_3	0	0	0
2b	500 mg L^{-1} GA_3	78 ± 1^{a}	15.56 ± 2^{a}	77 ± 1
3b	1000 mg L^{-1} GA_3	63 ± 1^{b}	17.78 ± 2^{ad}	64 ± 2
4b	2000 mg L^{-1} GA_3	70 ± 2^{c}	20 ± 0^{ae}	71 ± 2
5b	3000 mg $L^{-1}GA_3$	54 ± 1^{d}	24.44 ± 2^{ce}	55 ± 1
6b	0.5% KNO_3	0	0	0
7b	1% KNO_3	0	0	0
8b	1.5% KNO_3	63 ± 3^{b}	8.89 ± 2^{b}	62 ± 3
9b	2.0% KNO_3	0	0	0
10b	200 mg L^{-1} GA_3 + 0.5%KNO_3	63 ± 3^{b}	22.22 ± 2^{de}	61 ± 3
11b	500 mg L^{-1} GA_3 + 1% KNO_3	56 ± 1^{de}	31 ± 2^{f}	56 ± 2
12b	Water	0	0	0

T_{50}: time required to attain 50% germination, ±SEM (standard error of mean)
Note: For each treatment, means followed by the same letter in each column do not differ significantly at $p < 0.05$

produced no germination. Seeds treated with 200 mg L^{-1} GA_3 (Treatment 1b) did not germinate either but there was a positive linear relationship ($Y = 0.0076x + 4.4304$, $R^2 = 0.726$, $p = 0.03$) between germination percentage and increasing GA_3 concentration with a maximum germination of 24.44% for seeds treated with 3000 mg L^{-1} GA_3 ($T_{50} = 55$ days), Table 2. Increasing concentration of GA_3 decreased the time required to attain 50% germination (T_{50}) (Fig. 1), more so than the potted experiments (treatment 1a-11a). No seeds germinated following treatment with 0.5%, 1% or 2% KNO_3 (Treatment 6b, 7b and 9b) but, surprisingly, a small percentage of seeds (8.89%) did germinate following Treatment 8b (1.5% KNO_3). Neither 200 mg L^{-1} nor 0.5% KNO_3 alone (Treatments 1b and 6b respectively) produced any germination yet the combination of the two (Treatment 10b) did result in 22% germination. The highest germination percentage of 31% (T_{50} = 56 days) was obtained using a combination of 500 mg L^{-1} GA_3 and 1.0% KNO_3. Results from multiple comparison tests between the various treatments showed a significant difference in the mean germination percentage of seeds treated with a combination of 500 mg L^{-1} GA_3 + 1% KNO_3 with all concentrations of GA_3 and KNO_3 ($p < 0.05$) except for seeds treated in 3000 mg L^{-1} GA_3 and 200 mg L^{-1} + 0.5% KNO_3.

Seed viability

Embryos stained with tetrazolium indicated viability. Of the 150 seeds tested, 111 embryos (74%) were stained. Visual inspection of the embryos showed that they were curved with narrow cotyledons characteristic of 40% of Malesian *Elaeocarpus* species (Weibel 1968; Flora Malesiana Symposium 1989).

Seed weight and seedling growth

Seed weight ($n = 1440$) ranged from 160 to 630 mg (mean weight 385.58 ± 1.90 mg). The distribution was normal (Shapiro-Wilk test), with intermediate seeds constituting 74.93% of the total seed population followed by light and heavy that accounted for 16.31% and 8.75% respectively (Table 3). More heavy seeds (24.60%) germinated than intermediate (22.80%) or light seeds (19.57%), Table 3. There was no significant relationship between the seed weight and germination time ($p > 0.05$). The time to 50% germination (T_{50}) was longest in intermediate seed weight (218 days) and shortest for light seeds (188 days) (Table 3). One year after transplantation, 100% of seedlings emerging from heavy- and intermediate-weight seeds had survived but only 85% of those from light-weight seeds. Seeds < 320 mg had an average coat thickness of 0.66 mm compared with 1.42 mm for seeds > 480 mg. Light seeds had an average length of 12 mm whereas seed length in intermediate and heavy seeds measured an average 13 mm and 15 mm respectively. Similarly, seed width was lower in light weight seeds (7 mm) as compared to heavy seeds (8 mm), Table 3.

Significant differences ($p < 0.05$) were observed in the shoot height, leaf area and dry biomass of seedlings emerging from light seeds compared with those of intermediate and heavy seeds (Table 4). However, there was no significant difference between the intermediate and heavy seeds ($p > 0.05$).

Regression analysis showed that seed weight had a positive impact on seedling vigour with heavier seeds producing larger and heavier seedlings (Table 5).

Discussion

Seeds stored at 5 °C remained viable even after 12 months of storage indicating 'orthodox' (i.e. will survive

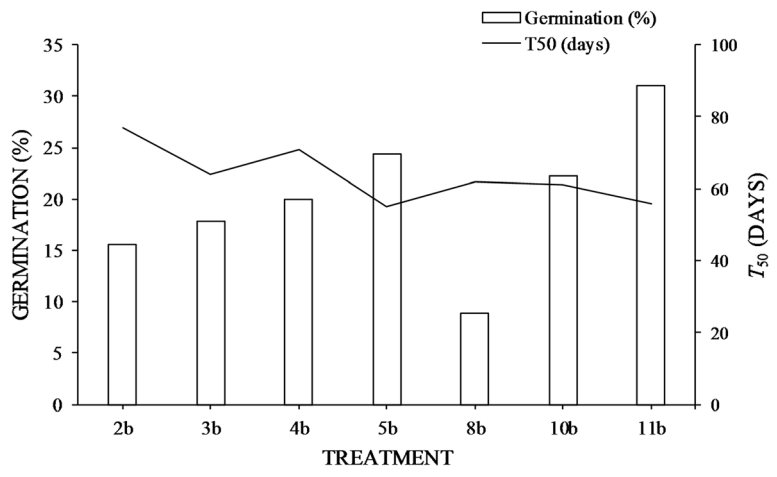

Fig. 1 Mean (±SEM) germination percentage (G%) and time to 50% germination (T_{50}) under GA_3 and KNO_3 treatments at 25 °C. (Treatments: 2b = 500 mg L^{-1}, 3b = 1000 mg L^{-1}, 4b = 2000 mg L^{-1}, 5b = 3000 mg L^{-1}, 8b = 1.5% KNO_3, 10b = 200 mg L^{-1} GA_3 + 0.5% KNO_3, 11b = 500 mg L^{-1} GA_3 + 1% KNO_3)

Table 3 Germination characteristics of *E. prunifolius* seeds

Parameters		Seed weight class(mg)		
		Light (< 320)	Intermediate (320–480)	Heavy (> 480)
Number of seeds sown (n)		235	1079	126
Average seed length (mm)		11.92 ± .064	13.47 ± .028	14.52 ± .059
Average seed width (mm)		6.80 ± .027	7.37 ± .017	8.02 ± .034
Number of seeds germinated		46	246	31
Proportion of Seed	Contribution to total seed lot (%)	16.31	74.93	8.75
	Germination (%)	19.57[a]	22.8[a]	24.6[a]
Mean germination (days)		211 ± 4	212 ± 1	213 ± 3
T_{50} (days)		188 ± 9	218 ± 7	204 ± 6
Seed coat thickness (mm)		0.66 ± 0.12	1.07 ± 0.06	1.42 ± 0.06
Weight of cotyledon (g)		0.077 ± 0.005	0.102 ± 0.002	0.117 ± 0.002

Note: For each treatment, means followed by the same letter in each row do not differ significantly at $p < 0.05$

drying and/or freezing during *ex situ* conservation) characteristics (Chin et al. 1989). However, intact untreated seeds took an average of 213 days to germinate under favourable conditions, which clearly indicates the presence of dormancy (Baskin and Baskin 1998, 2004). Physical and mechanical dormancy is the most common inhibitor of germination in many species. Threatened tree species such as *Cupressus atlantica* (Youssef et al. 2012), *Elaeocarpus blascoi* Weibel (Ramasubbu and Irudhyaraj 2016) and *Intsia bijuga* (Colebr.) Kuntze (Thaman et al. 2006) exhibit exogenous dormancy where the hard seed coats inhibit water penetration that is required for embryo growth and development.

Reducing germination time and increasing germination percentage are both important pre-requisites of a successful propagation initiative. Cracked *E. prunifolius* seeds germinated faster and in greater numbers than intact seeds, as expected by disrupting the hard coat surrounding the seed. Similar results have been reported in *E. ganitrus* (Khan et al. 2003). However, cracked seeds still remained dormant for ca. 3 months and the germination time decreased further only by soaking in gibberellic acid solution ($\geq 500\ mg\ L^{-1}$) or a combination of gibberellic acid and potassium nitrate. Similar results have been reported in species like *Ramonda serbica* Pančić, *R. nathaliae* Pančić & Petrovič, *Magnolia yunnanensis* (Hu) Noot. and *M. punduana* (Hk. f & Th.) Figlar (Gashi et al. 2012; Han et al. 2010; Iralu and Upadhaya 2016). The presence of abscisic acid (ABA) in mature seeds has been associated with seed dormancy (Matakiadis et al. 2009; Hilhorst and Karssen 1992), and studies have established that the application of nitrate ions to a germination medium leads to rapid decline of ABA. However, three of the four concentrations of potassium nitrate tested alone in the current study prevented germination. The positive effect of some PGAs in the current study indicates that (in addition to physical dormancy) seeds may also have 'physiological' dormancy also called 'combined' dormancy (Baskin and Baskin 1998; Nikolaeva 1969), which was broken by the

Table 4 Shoot height and root length (cm), number of leaves/plant, leaf area (cm^2) and dry weight/plant (g) of seedlings of *E. prunifolius* (±SEM, $n = 10$) in light-, intermediate-and heavy-seed weight classes grown under three different contrasting light regimes after 1 year of transplantation

Day length (h)/light intensity (mol m^{-2} d^{-1})	Seed weight (mg)	Shoot height (cm)	Root length (cm)	Number of leaves/plant	Leaf area/seedling (cm^2)	Dry weight/plant (g)
10/16	< 320	20.57 ± .39[a]	16.63 ± 0.54[a]	7.2 ± 0.48[a]	9.93 ± 0.18[a]	0.56 ± 0.08[a]
	320–480	39.25 ± 0.90	26.80 ± 1.10	15.4 ± 0.52[a]	21.09 ± 0.69	2.76 ± 0.67
	> 480	26.46 ± 0.17[a]	17.28 ± 0.35[a]	11.4 ± 0.39[a]	13.72 ± 0.74[a]	1.43 ± 0.33[a]
10/4	< 320	31.27 ± 1.07	25.84 ± 2.13[b]	12.40 ± 0.25[b]	15.81 ± 0.63	1.74 ± 0.24
	320–480	46.68 ± 2.66[b]	26.22 ± 0.72[b]	15.6 ± 1.28[c]	22.61 ± 1.03[b]	2.95 ± 1.12[b]
	> 480	42.81 ± 0.71[b]	30.63 ± 1.42[b]	13.06 ± 0.68[bc]	24.48 ± 1.37[b]	3.31 ± 0.48[b]
10/1	< 320	9.70 ± 1.18[c]	5.82 ± 2.70[c]	3 ± 0.40	3.63 ± 1.15[c]	0.06 ± 0.15[c]
	320–480	18.96 ± 0.42[d]	21.52 ± 1.19[d]	7.4 ± 0.76[d]	10.42 ± 0.83[d]	0.27 ± 0.19[c]
	> 480	15.15 ± 0.46[cd]	13.80 ± 1.83[cd]	6.8 ± 0.17[d]	7.44 ± 0.53[cd]	0.19 ± 0.22[c]

Note: For each treatment, means followed by the same letter do not differ significantly at $p < 0.05$ (Tukey's multiple range test)

Table 5 Seed weight influence on seedling characteristics in *E. prunifolius*. Regression Eq. ($Y = c + mx$) for the relationship between initial seed weight ($n = 10$), shoot and root length (cm), number of leaves/plant, leaf area (cm^2) and dry weight/plant (g)

Variables	Regression equation	*r*	*p* value
Shoot height	$Y = 19.722 + 51.963$	0.531	0.002
Root length	$Y = 16.401 + 28.830$	0.530	0.002
Number of leaves/plant	$Y = 10.814 + 7.838$	0.313	0.091
Leaf area	$Y = 6.276 + 37.577$	0.791	0.000
Dry biomass	$Y = -0.540 + 8.284$	0.763	0.000

Significant at $p < 0.05$

application of gibberellic acid (Baskin and Baskin 1998, 2004). Physiological dormancy has also been reported from other species of *Elaeocarpus* such as *E. floribiindus*, *E. petiolatm* and *E. stipularis* (Ng 1978, 1980; Beniwal and Singh 1989).

The fruits of *E. prunifolius* mature in early September and remain dormant throughout the winter (November–February). The seeds germinate in April with the rise in temperature and onset of rain indicating that these environmental cues may be a requirement for germination. Similar moisture-aided germination has been reported in *Prunus jenkinsii* Hook. f. & Th. and other endemic species of the region (Upadhaya et al. 2007; Upadhaya et al. 2017). However, uncracked seeds imbibed only a small amount of water during soaking and this was not improved by pre-cracking the seeds. Soaking either cracked or uncracked seeds in water before sowing might be expected to improve the speed and extent of germination but, surprisingly, resulted in no germination. In contrast, Khan et al. (2003) found that soaking *E. ganitrus* seeds in hot water for 24 h then fermenting them for 20 days led to 37% germination. All the sulphuric acid treatments test in the current study prevented germination of *E. prunifolius* seeds yet 15% of *E. serratus* seeds treated with 50% nitric acid germinated (Dahanayake et al. 2013).

Seed-coat impermeability is usually associated with the presence of one or more layers of impermeable palisade layers of lignified cells (Corner 1976; Vazquez-Yanes and Perez-Garcia 1976). However, seed coat thickness did not impede or delay germination as germination percentage was higher in heavy seeds (which had thicker coats) and the mean germination time was not significantly different across the seed weight classes. Similar findings have been observed in *Oenothera biennis* L (Gross and Kromer 1986), *Diplotaxis erucoides* (L.) DC. and *D. virgata* (Cav.) DC. (Perez-Garcia et al. 1995). Barnett (1997) observed that 69% of the variation in the speed of germination in five pine species was related to the seed coat. In the present study, germination percentage was positively correlated with seed weight. This finding is similar to that reported in *Artocarpus heterophyllus* L., *Alangium lamarckii* Thwaites, *Quercus semiserrata* Roxb. and other oak species (Khan 2004; Ahirwar 2012; Barik et al. 1996; Bonfil 1998; Tripathi and Khan 1990; Khan and Shankar 2001). Heavy seeds are associated with greater stocks of food and energy reserves and provide readily available energy and resources to stimulate germination (Flint and Palmblad 1978).

The seedlings from heavy seeds survived better and exhibited greater biomass but the amount of heavy seed in the sample tested was low. Similar results have been observed in *Quercus* species (Tripathi and Khan 1990; Khan and Shankar 2001). The current study was part of a larger project to examine issues with *E. prunifolius* germination in the wild (data not shown). The main constraint of germination under natural conditions was premature seed fall caused by strong winds and rain. Also, mature fruits were predated by rodents, birds, worms and ants before the seeds germinated. A substantial number of seeds did germinate but, young seedlings suffered mortality due to desiccation during winter and also due to trampling. Similar observations have also been reported in other *Elaeocarpus* species (Matthew 1999).

Conclusion

Elaeocarpus prunifolius seed exhibits both physical and physiological dormancy. Splitting the seed coats with a vice is a prerequisite for propagation and is also a cost-effective method. In addition, treating cracked seeds with a combination of GA_3 and KNO_3 accelerates the germination rate, which may enable mass propagation for reintroduction programmes. Heavy seeds showed better survival and growth than lighter seeds so heavy seeds should be separated out wherever practical and used for propagation.

Abbreviations

ABA: Abscisic acid; GA_3: Gibberellic acid; H_2SO_4: Sulphuric acid; $KMnO_4$: Potassium permanganate; KNO_3: Potassium nitrate; T_{50}: Time to 50% germination

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Authors' contributions

VI and KU conceived and designed the study. Experimental works were carried out by VI. Data analysis was done by KU and manuscript was drafted by VI and KU. Both authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

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