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Morphophysiological epicotyl dormancy in seeds of three *Psychotria* species from Sri Lanka: first record for Rubiaceae

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Abstract

To increase our knowledge of the diversity of seed dormancy and germination in Rubiaceae, we investigated seed desiccation sensitivity and germination of three *Psychotria* species. Seeds of *P. gardneri*, *P. nigra* and *P. zeylanica* germinated to high percentages at <15% seed moisture content. Intact seeds of *P. zeylanica* and *P. nigra* imbibed water and thus do not have physical dormancy. More than 50% of the seeds of *P. zeylanica*, *P. nigra* and *P. gardneri* took 33, 53 and 110 d, respectively, at 25°C for the radicle to emerge, and embryo growth occurred before and after radicle emergence. Thus, seeds have morphophysiological dormancy. Shoot emergence of *P. nigra* and *P. zeylanica* seeds was delayed 50 and 80 d after radical emergence, respectively; thus, seeds have epicotyl morphophysiological dormancy (eMPD). This is the first report of eMPD in Rubiaceae. Since warm stratification promoted both radicle and shoot emergence in seeds of *P. zeylanica* and *P. nigra*, the level of eMPD is non-deep simple. Hence, dormancy of the studied *Psychotria* spp. can be described as $C_{1b}B_b$ (radicle)–$C_{1b}B_b$ (epicotyl), i.e. the embryo is underdeveloped and grows prior to radicle emergence and epicotyl emergence under warm temperatures (B_b), and both the radicle and epicotyl have non-deep simple physiological dormancy broken by warm temperatures (C_{1b}). In two *Psychotria* species studied in detail, radicle emergence occurs at the beginning of the rainy season and plumule emergence at the peak rainy season when conditions are most favourable for rapid seedling development.

Keywords: embryo growth, germination requirements, physiological dormancy, seed desiccation sensitivity, seed dormancy

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Introduction

Information on the kinds of seed dormancy (and non-dormancy) within each plant family would greatly facilitate investigations on the evolutionary origins and relationships of the five classes of dormancy (and non-dormancy) (Baskin and Baskin, 2014). For some plant families such as the Cannaceae, Cistaceae and Nelumbonaceae, there seems to be no variation, and all species have physical dormancy. In contrast, the Fabaceae and Malvaceae have three classes of dormancy (physical, physiological and combinational) as well as some species having non-dormant seeds (Baskin and Baskin, 2014). For many other families, such as the Rubiaceae, we do not have a comprehensive overview of the diversity of dormancy classes within the family. Many species of Rubiaceae are reported to have seeds with physiological dormancy, but Baskin and Baskin (2014) caution that further research on members of the Rubiaceae may reveal greater diversity than has been ascribed previously to the family.

There are two reasons for thinking that some members of the Rubiaceae may have a class(es) of dormancy in addition to physiological dormancy. (1) The embryo in *Coffea arabica* seeds is differentiated and consists of a radicle, axis and two small cotyledons (Da Silva *et al*., 2008). Furthermore, De Farias *et al*. (2014) reported that the embryo in C. *arabica* seeds grows prior to radicle emergence, and that it does so within 9 d following imbibition. (2) Drawings of seeds of a few species of Rubiaceae, e.g. *Mitchella repens, Psychotria tenuifolia* (Martin, 1946) and *P. griffithii* (Ng, 1992), have been published, showing small, presumably underdeveloped embryos. If these small embryos must grow prior to radicle emergence and if growth and germination occur within less than about 1 month, seeds would have morphological dormancy. However, if embryos must grow prior to radicle emergence and if germination takes longer than about 1 month and dormancy-breaking treatments are required, seeds

would have morphophysiological dormancy (Baskin and Baskin, 2014). On the other hand, the small embryos may not grow prior to radicle emergence, e.g. some Nymphaeaceae (Baskin and Baskin, 2007).

Psychotria (Rubiaceae) is the third largest genus of angiosperms, consisting of more than 1800 species (Sohmer, 1988; Davis *et al.*, 2001a; Mabberley, 2008). It is a pantropical genus contributing significantly to the floristic diversity of the tropics (Nepokroeff *et al.*, 1999), and a significant portion of the understorey of wet tropical forests consists of *Psychotria* spp. (Sohmer, 1988). Thus, *Psychotria* has been used widely as a model genus for studying patterns of speciation, ecological mechanisms and reproductive systems among tropical plants (Hamilton, 1989). Furthermore, since *Psychotria* is one of the important genera of the understorey tree layer of tropical wet and tropical montane forests in Sri Lanka (Dissanayake, 1987), as well as in other tropical areas, it is one of the significant genera that can be used to restore degraded forests (Olson and Dinerstein, 1998). Information on seed germination, seed dormancy and storage behaviour are important for production of seedlings for restoration activities. Studies on about 25 species of *Psychotria* indicate that seeds are slow to germinate (Baskin and Baskin, 2014). For example, *P. leiocarpa* seeds took approximately 3 months to germinate (Henriques *et al.*, 2004), and those of *P. hoffmansegiana* remained in the soil >3 months after dispersal before germinating (Araújo and Cardoso, 2007). Thus, it is clear that seeds of various species of *Psychotria* are dormant, but no attention has been given to embryo morphology and to the possibility that seeds could have morphological or morphophysiological dormancy (MPD). Based on (1) what appear to be underdeveloped embryos in seeds of *Psychotria tenuifolia* (Martin, 1946) and *P. griffithii* (Ng, 1992) and (2) dormancy (slowness to germinate) in seeds of various species of *Psychotria*, we hypothesized that seeds of the *Psychotria* species in Sri Lanka would have MPD.

Fourteen *Psychotria* species have been recorded in Sri Lanka, and the majority of them are found in the wet zone of the country. Eleven of the Sri Lankan *Psychotria* spp. are endemics (Dissanayake, 1987; MOE, 2012), and eight of them are categorized as nearly threatened, vulnerable or endangered species on the red data list of threatened species (MOE, 2012). On the other hand, the genus *Psychotria* is characterized by the presence of many economically important chemicals, including some with high pharmacological activities (Adjibade *et al.*, 1992; Judd, 1999; Davis *et al.*, 2001b; Henriques *et al.*, 2004; Lops *et al.*, 2004; Corrêa *et al.*, 2008). However, the habitats of most *Psychotria* spp. are limited in Sri Lanka and are threatened by various anthropogenic and natural causes (Gunatilleke and Gunatilleke, 1990). Thus, there is a threat of extinction for Sri Lankan *Psychotria* spp., which have high ecological and economic potential. Clearly, there is an immediate need to undertake the necessary conservation measures to protect the species of this valuable genus. Since seeds play an important role in the persistence of plant populations (Vazquez-Yanes and Orozco-Segovia, 1993) and in the production of saplings for restoration and regeneration activities (Engel and Parrotta, 2001; Baskin and Baskin, 2005), knowledge of seed sensitivity to drying, dormancy, dormancy-breaking treatments and germination requirements is crucial in planning conservation activities.

Rubiaceae is a family showing high diversity when considering seed desiccation tolerance. Intermediate sensitivity to desiccation was first observed in seeds of *Coffea* spp. (Ellis *et al.*, 1990). Furthermore, desiccation-sensitive and desiccation-tolerant seed-producing species have been observed among the Rubiaceae (Ellis *et al.*, 1990; Dussert *et al.*, 1999; Orwa *et al.*, 2009). Knowledge on sensitivity of seeds to desiccation is very important as it assists in determining the proper storage conditions for long-term seed storage (Hong and Ellis, 1996).

Within the context of expanding our knowledge of the diversity of dormancy classes in plant families, and in Rubiaceae in particular, seed dormancy was studied in three species of *Psychotria* (*P. gardneri, P. nigra* and *P. zeylanica*) from the montane zone of Sri Lanka. The broad objective of our research on these three species was to determine the seed desiccation sensitivity and dormancy-breaking and germination requirements. All three species are rare and ecologically important in their montane habitats; thus, knowing how to germinate and store seeds of these species would benefit conservation efforts.

Materials and methods

Seed collection sites and the study species

Fruits for the experiments were collected from Knuckles, Horton Plains and Hanthana Mountains in Sri Lanka. Knuckles Forest Reserve is in the Knuckles Mountains which lie between latitudes 7°18′–7°34′N and longitudes 80°41′–80°55′E. The altitudes of these mountains range from 900 to 2000 m, with approximately 21,000 ha of forest cover. The montane forest zone where seeds were collected is extremely wet during most months with a mean annual rainfall of about ~5000 mm (Bambaradeniya and Ekanayake, 2003), and the mean monthly temperature varies between 15 and 25°C (Weerawardhena and Russell, 2012). Horton Plains is the highest peneplain in Sri Lanka. It is located at latitude 6°48′N and longitude 80°48′E and covers 3160 ha of the central highlands, with elevations ranging from 2100 to 2300 m. Mean annual rainfall of the area is about 2150 mm, and the mean annual

temperature in the montane zone is about 15°C, with temperatures varying between 5 and 27°C (Green, 2008). The Hanthana Mountains, with elevations ranging from 800 to 1200 m, are located at latitude 7°16′N and longitude 80°37′E and cover 432 ha in the Kandy District in Sri Lanka. The mean annual temperature in the mountain area is about 24.1°C, and the mean annual precipitation is about 2000 mm.

Psychotria nigra (Gaertn.) Alston is a shrub growing up to 4 m tall and is native to South Asia, including India and Sri Lanka. Usually, plants can be found in the understorey of wet forests at elevations >800 m in Sri Lanka. *P. nigra* fruits are globose drupes that are ~9 mm long (Dassanayake, 1987). They were collected from Knuckles Forest Reserve and from the Hanthana Mountains. Those collected from Knuckles Forest Reserve were used for the seed desiccation experiment, and those collected from the Hanthana Mountains were used for seed dormancy experiments.

Psychotria zeylanica (Sohmer) is an endemic shrubby tree growing up to 3 m tall, found in the understorey of the wet forests in the central highlands of Sri Lanka, usually above 1500 m elevation. Mature fruits (drupes) are obovoid–ellipsoid, black and 9–11 mm long (Dassanayake, 1987). They were collected from Horton Plains.

Psychotria gardneri (Thw.) Hook. f. (var. *gardneri*) is a slender, much branched and glabrous tree, growing up to 5 m tall. This species is endemic to Sri Lanka and can be found in the subcanopy of the middle-elevation forests to about 1600 m (Dassanayake, 1987). Fruits (drupes) of this species are globose and ~6 mm in diameter, and they were collected from the Hanthana Mountains.

Dispersal units, ripe fruits (dark blue to black coloured and fleshy) were collected from five or more individuals of each species from each site. Fruits were collected at fruiting peak at each site for each species. Fruits of *P. gardneri* were collected on 13 January 2012 and 31 July 2013, *P. nigra* on 31 July 2013 and *P. zeylanica* on 8 February and 7 December 2012. Fruits were collected directly from the trees, placed in polythene bags, tagged and transported to the Department of Botany, University of Peradeniya, where the laboratory experiments were conducted. Seeds (enclosed by the endocarp, hereafter seeds) were removed from the fruits, washed, air dried and stored in plastic bottles at room temperatures until used. Laboratory experiments were initiated within 3 d after seed collection.

Seed desiccation sensitivity

The purpose of these experiments was to determine the level of desiccation sensitivity of seeds of the study species.

Seed moisture content (MC)

The mass of 15 fresh seeds (just after extraction of seeds from the mesocarp) for each species was measured individually using a digital balance to the nearest 0.0001 g. Then, seeds were dried at 120°C for 3 h (ISTA, 2008). After drying, the mass of each seed was re-measured. Initial MC was calculated on a fresh mass basis using initial mass and dry mass.

Effect of drying on seed viability

Three replicates of 15 seeds each for *P. nigra* and *P. gardneri* were dried to 14 and 12% MC, respectively, by keeping the seeds in a silica-gel desiccator for about 1 week. Dried seeds of *P. nigra* and *P. gardneri* were incubated in 9-mm-diameter Petri dishes (hereafter 'Petri dishes') on eight layers of tissue paper [Flora paper serviette product code A1101, Pee Bee Management Services (Pvt.) Ltd. Homagama, Sri Lanka] moistened with distilled water at 25°C in light/dark (14 h/10 h, hereafter 'light') for 3 and 5 months (completed germination), respectively. At the end of the experiment, all non-germinated seeds were dissected (cut-test) to check the viability of the embryo (white and firm, a viable embryo vs. dark and soft, a non-viable embryo). Germination of dried seeds was compared to that of non-dried seeds, using a standard germination test (described under the heading 'Standard germination (control)'), to determine the effect of drying on seed viability.

Seed dormancy

The purpose of these experiments was to determine if seeds are dormant and, if so, to identify the class and level of dormancy. Results from the germination and imbibition tests, changes in embryo:seed length (E:S) ratio, responses to gibberellic acid (GA_3), and temperature and time required for radicle and shoot emergence were used to determine the class and level of dormancy.

Imbibition test

The initial mass of 15 fresh intact and 15 manually scarified seeds of *P. gardneri* and of *P. zeylanica* were measured individually using a digital chemical balance to the nearest 0.0001 g. Seeds were placed separately on eight layers of moistened tissue paper in Petri dishes and incubated at ambient laboratory conditions. Seeds were retrieved after 2, 5, 7 and 24 h and then after 4, 11, 19 and 26 d, weighed and returned to the Petri dishes. The average percentage mass increase during imbibition was plotted against time. The final mass increase of seeds was defined as the mass

increase at the time the imbibition curve reached a plateau.

Standard germination (control)

Three replicates of 15 fresh intact seeds of each species at initial MC were incubated in Petri dishes on moistened tissue paper in light at 25°C. Seeds were checked for germination at 3-d intervals for 3 months for *P. zeylanica* and *P. nigra* (none of seeds germinated after 75 d) and 5 months for *P. gardneri* (after 5 months all non-germinated seeds were rotten). Radicle emergence (>1 mm) was the criterion for germination. At the end of the experiment, all non-germinated seeds were dissected (cut-test) to check the viability of the embryo. GT_{50} (time taken for 50% germination) was calculated for each species, as explained under 'Data analysis' below.

Embryo length:seed length ratio (E:S ratio)

The embryo length:seed length ratio was determined for ten freshly collected seeds of each species and for ten seeds of each species at the time the endocarp split (but before the length of the emerging radicle exceeded 0.5 mm), indicating that the seed was germinating. Freshly collected seeds were allowed to imbibe for 24 h, and then the length of the seed (S) and embryo (E) of each was measured using a Vernier caliper. When the E:S ratio was determined in endocarp-split seeds, the embryo was measured excluding the portion outside the seed (i.e. the excluding radicle). The E:S ratio was calculated to determine the development of the embryo prior to radicle emergence, and the percentage embryo development prior to radicle emergence was calculated using the following formula:

$$\text{Percentage embryo development} = \frac{\text{Average E:S ratio of seeds soon after endocarp rupture}}{\text{Average E:S ratio of fresh seeds}} \times 100.$$

Furthermore, embryo and seed length of *P. gardneri* were monitored continually at 7-d intervals until plumule emergence occurred, after 106 d.

Gibberellic acid treatment

Three replicates of 15 seeds each of the three species were incubated in Petri dishes on eight layers of tissue paper moistened with 100 ppm and 500 ppm gibberellic acid (GA_3) solutions in light at 25°C. Germination was checked at 3-d intervals for 90 d, and radicle emergence was the criterion for germination. The standard germination test, as explained above, was used as the control; these tests were initiated at the same time. GT_{50} was calculated for each treatment for each species.

Effect of temperature on seed germination

Three replicates of 15 fresh *P. nigra* seeds were incubated in Petri dishes on eight layers of tissue paper moistened with distilled water in light at 8, 15, 25 and 35°C, and three replicates of 15 fresh *P. zeylanica* seeds were incubated in light at 15, 25 and 35°C. Seeds were checked for germination at 3-d intervals for 90 d, and radicle emergence was the criterion for germination. Furthermore, three replicates of 15 fresh *P. nigra* seeds were incubated at 8, 15, 35°C for 2 weeks and then transferred to 25°C. Similarly, three replicates of 15 fresh *P. zeylanica* seeds were incubated at 35, 35, 25, 15 and 8°C for 2 weeks, after which seeds were transferred to 15, 25, 15, 25 and 25°C, respectively. Seeds were observed for germination at 3-d intervals for 90 d after the transfer to the second set of temperatures. These temperatures were chosen considering the mean temperatures in the habitats of these species at different times of the year (Green, 2008; Weerawardhena and Russell, 2012). GT_{50} was calculated for each treatment for each species.

Epicotyl dormancy

Fifteen radicle-emerged (in light at 25°C) seeds each of *P. zeylanica* and *P. nigra* were incubated on 24 layers of tissue paper moistened with distilled water in a 1000-ml beaker in light at 25°C. Seeds were marked individually. The time taken for the shoot (plumule) to emerge from each seed was recorded. In addition, 15 radicle-emerged (in light at 15°C) seeds of *P. zeylanica* were incubated on tissue papers moistened with distilled water in light at 15°C in a 1000-ml beaker. Furthermore, 15 radicle-emerged seeds of *P. nigra* were incubated on tissue papers moistened with 100 ppm GA_3 solution in a 1000-ml beaker in light at 25°C. Seeds were observed at 3-d intervals for plumule emergence, and the time taken for plumule emergence of each seed was recorded.

Morphological development of the plumule

Sixty seeds of *P. gardneri* were incubated on tissue papers moistened with water in light at 25°C. Ten seeds each were retrieved after 1, 2, 4, 6, 12 and 15 weeks, and the E:S ratio was determined as described above. Furthermore, seed and embryo lengths of *P. zeylanica* and *P. nigra* seeds were measured just after the endocarp ruptured and just before cotyledon emergence.

Data analysis

As binary logistic regression analysis is more appropriate to analyse experiments with two possible outcomes, germination data (two outcomes: germinated or not germinated) were statistically analysed with

this procedure using Minitab 16.0 statistical software (Minitab® 16.2.3, Minitab Inc., State College, Pennsylvania, USA). Weibull 4 parameter sigmoidal curves were fitted to the germination time courses (as suggested by Brown, 1987) using Sigmaplot 10.0 software (Sigmaplot for Windows version 10.0, Dundas software Ltd, ©wpcubed GmbH, Erkrath, Germany), to identify the germination patterns. GT_{50} of each treatment was determined, using the fitted curve, as the time until half-maximal germination. As normality and the homoscedasticity of the water imbibition, E:S ratio and time taken for plumule emergence data were confirmed by an Anderson–Darling test and by Bartlett's and Levene's tests, respectively, pooled *t*-tests were used to check the similarity or dissimilarity of water imbibition patterns of non-scarified and scarified seeds and the E:S ratios of non-germinated and germinated seeds. One-way analysis of variance (ANOVA) was used to analyse the time taken for plumule emergence and E:S ratios of seeds in different treatments (e.g. temperature conditions, different GA treatments) as there were more than two treatments for these experiments.

Results

Seed desiccation sensitivity

Seed moisture content

The fresh mass of *P. zeylanica, P. nigra* and *P. gardneri* seeds was 0.030 ± 0.005, 0.079 ± 0.013 and 0.017 ± 0.002 g, respectively, while the dry mass was 0.025 ± 0.004, 0.049 ± 0.008 and 0.013 ± 0.001 g, respectively. Thus, the initial MC of *P. zeylanica, P. nigra* and *P. gardneri* seeds was 15.9, 38.2 and 24.5%, respectively.

Effect of drying on seed viability

Under the initial MC, all the viable seeds of *P. nigra* and *P. gardneri* germinated during 90 and 144 d, respectively. When seeds of *P. nigra* and *P. gardneri* were dried to 14 and 12% MC, respectively, their germination percentage was not significantly different from that of the control (Table 1).

Seed dormancy

Imbibition test

After 26 d, the mass increase of non-scarified *P. zeylanica* and *P. gardneri* seeds on tissue papers moistened with distilled water was 55.9 ± 6.5 and 52.8 ± 11.2%, respectively, while the mass increase of scarified seeds of *P. zeylanica* and *P. gardneri* was 56.0 ± 12.2 and 53.1 ± 11.6%, respectively. However, no significant differences were observed between increase in mass of non-scarified and manually scarified seeds of *P. zeylanica* ($t = -0.87$, $P = 0.40$) and of *P. gardneri* ($t = -0.08$, $P = 0.93$) after imbibition for 26 d.

Standard germination (control)

Seeds of *P. zeylanica* first showed radical emergence after about 15 d of incubation, while those of *P. nigra* and *P. gardneri* took 33 and 59 d, respectively (Fig. 1). After 90 d, seeds of *P. zeylanica, P. nigra* and *P. gardneri* had germinated to 77.3, 53.3 and 13.3%, respectively; after 144 d only 55.6% of the *P. gardneri* seeds had germinated. Non-germinated *P. nigra* and *P. zeylanica* seeds were non-viable by 90 d, while all non-germinated *P. gardneri* seeds were non-viable by 150 d. The time for *P. zeylanica, P. nigra* and *P. gardneri* seeds to reach 50% germination (GT_{50}) was 33, 53 and 110 d, respectively.

Embryo length:seed length ratio (E:S ratio)

The E:S ratio of *P. gardneri* seeds at maturity and soon after the endocarp ruptured (after about 72 d of incubation) was 0.44 ± 0.07 and 0.69 ± 0.08, respectively. Embryo development (E:S ratio increment) reached 56.8% during this period. Similarly, the E:S ratio of the other two species increased during incubation prior to radicle emergence (Table 2, Fig. 2). Further,

Table 1. Initial moisture content (MC) of *P. zeylanica, P. nigra* and *P. gardneri* seeds (mean seed MC ± 1 SD) and MC after seeds were dried in a silica-gel desiccator (Low MC), and germination percentage of seeds at each MC. The *P* values were calculated from binary logistic regression and compared the germination of each species at their initial MC with germination after drying. $P < 0.05$ indicates significant differences between germination at the initial MC vs. that at the MC after drying

		Initial MC		Low MC		
Species	Location	MC (%)	Germination (%)	MC (%)	Germination (%)	*P* value
P. zeylanica	Horton plains	15.9 ± 0.7	77.3 ± 10.1			
P. nigra	Hanthana	35.5 ± 2.9	53.3 ± 13.3			
	Reverston	38.2 ± 7.3	75.6 ± 19.3	14.0 ± 0.5	77.7 ± 4.0	0.80
P. gardneri	Hanthana	24.8 ± 3.4	55.6 ± 13.8	12.0 ± 0.8	60.0 ± 13.3	0.67

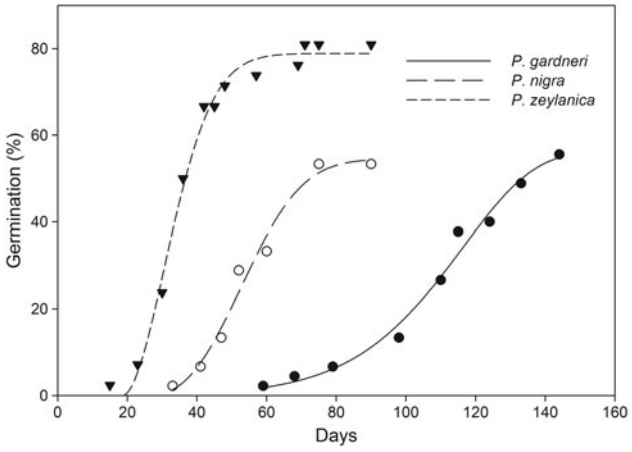

Figure 1. Radicle emergence of intact freshly collected seeds of *P. gardneri, P. nigra* and *P. zeylanica* incubated at 25°C in light/dark (14/10 h). Weibull 4 parameter sigmoidal curves were used to predict germination.

since embryo growth of *P. gardneri* continued until plumule emergence, the E:S ratio increased significantly between the time of endocarp rupture and plumule emergence (at 106 d of incubation) ($F = 52.67$, $P < 0.001$).

Gibberellic acid treatment

The percentage germination of *P. nigra* seeds was increased by 100 ppm GA_3, but treatment with GA_3 had no effect on germination of the other two species (Table 3). However, first germination of *P. nigra* and *P. gardneri* seeds was observed earlier than that of the control when they were treated with either concentration of GA_3.

Effect of temperature on seed germination

The highest germination of *P. nigra* seeds (53.3%) was observed at 25°C, and none of the seeds germinated at 8, 15 or 35°C. All the temperature changes caused a delay in the time to first germination of *P. nigra* seeds compared to that of the control. In *P. zeylanica*, 60.0% of the seeds germinated at 25°C, 16.7% germinated at 15°C and none germinated at 35°C. Temperature alterations did not significantly increase the final germination percentage of *P. zeylanica* seeds compared to that of the control at constant 25°C. Except for 15 → 25°C and 8 → 25°C, temperature changes decreased the germination percentage compared to that of the control (Fig. 3).

Epicotyl dormancy

At 25°C, an average of 54.1 ± 8.6 and 134.4 ± 10.5 d were required for the beginning of radicle and plumule emergence, respectively, for *P. zeylanica* seeds. Furthermore, 53.1 ± 8.6 and 135.7 ± 12.0 d were required for the beginning of radicle and plumule emergence, respectively, when seeds were incubated at 15°C. No significant effect of temperature was observed on either time or percentage of plumule or radicle emergence from *P. zeylanica* seeds. At 25°C on tissue papers moistened with distilled water, the radicle and plumule emerged from *P. nigra* seeds after 42.6 ± 14.8 and 92.2 ± 28.5 d, respectively; plumule emergence was first observed after 54 d. However, radicles emerged from *P. nigra* seeds within 30.5 ± 5.4 d when seeds were incubated on tissue papers moistened with GA_3 solutions. Seeds incubated on GA_3 solutions did not show first plumule emergence until 61 d from the date of incubation. Unfortunately, no observations were made after 61 d, since all the emerged radicles died due to dehydration of the medium.

Morphological development of the plumule

Seeds of the three species contained a linear embryo, and they showed epigeal germination. The E:S ratio of *P. gardneri* seeds, which was 0.44 ± 0.07 initially, increased to 0.69 ± 0.08 by the time of endocarp rupture and continued to increase to 0.95 ± 0.12 by the time of plumule emergence (Fig. 4). The E:S length ratio of the other two species also increased with a similar

Table 2. Development of embryo, i.e. embryo length:seed length ratio (E:S), from time of dispersal until radicle and plumule emergence of *P. gardneri*. *P. zeylanica* and *P. nigra* seeds (mean seed E:S ± 1 SD). *P* value < 0.05 indicates significant differences between E:S ratio at dispersal maturity and at the time of germination (radicle emergence)

		Soon after endocarp rupture			Soon after plumule emergence	
Species	E:S ratio at time of dispersal	E:S ratio	Embryo development (%)	Statistical parameters	E:S ratio	Embryo development (%)
P. zeylanica	0.36 ± 0.04	0.49 ± 0.09	36.1	$P = 0.003$ $t = -3.83$	1.00 ± 0.00	172.2
P. nigra	0.43 ± 0.04	0.60 ± 0.08	39.5	$P = 0.029$ $t = -3.92$	1.00 ± 0.00	125.6
P. gardneri	0.44 ± 0.07	0.69 ± 0.08	56.8	$P = 0.001$ $t = -5.79$	1.00 ± 0.10	120.4

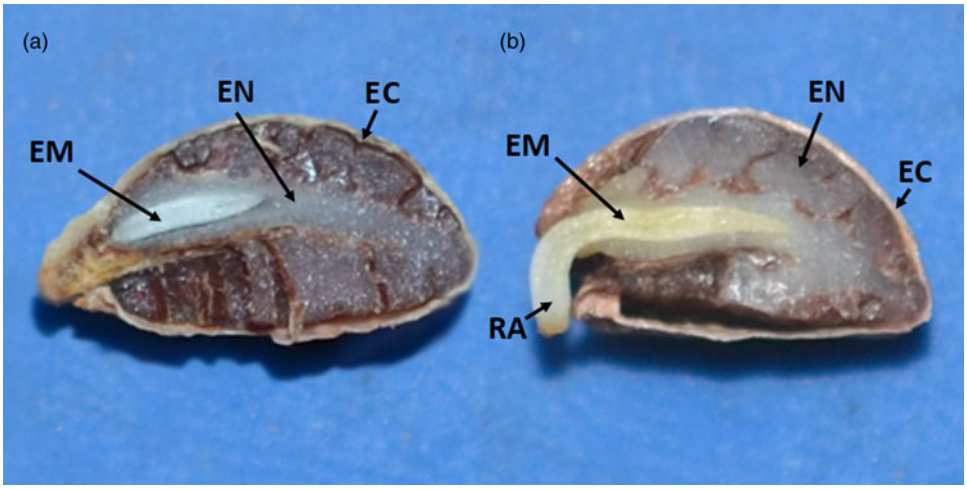

Figure 2. Longitudinal cross-section of *P. nigra* seed: (a) freshly matured seed and (b) just after radicle emergence. EC, endocarp; EM, embryo; EN, endosperm; RA, emerged radicle.

Table 3. Germination (mean % ± SD) and days to first germination of *P. zeylanica, P. nigra* and *P. gardneri* seeds on filter papers moistened with 100 ppm or 500 ppm gibberellic acid (GA_3) solutions or with distilled water (as the control)

	P. zeylanica			*P. nigra*			*P. gardneri*		
		Germination (%)			Germination (%)			Germination (%)	
Treatment	Days to first germination	after 30 d	Total	Days to first germination	after 30 d	Total	Days to first germination	after 30 d	Total
Control	15	22.2 ± 16.6	77.3 ± 10.1	33	0.0	53.3 ± 13.3	59	0.0	55.6 ± 13.9
$GA_3$100 ppm	23	22.2 ± 10.2	77.8 ± 10.2	21	6.7 ± 5.8	76.7 ± 11.6	40	0.0	35.6 ± 34.2
$GA_3$500 ppm	23	20.0 ± 0.0	60.0 ± 11.5	21	6.7 ± 5.8	33.33 ± 11.54	40	0.0	40.0 ± 6.7

Germination of all species after 30 d under GA_3 treatments was not significantly different from that of the control ($P_{P.\ nigra} = 0.100$, $P_{P.\ zeylanica} = 0.946$). Similarly, total germination of *P. zeylanica* and *P. gardneri* seeds in the GA_3 treatment was not significantly different from that of the control ($P_{P.\ zeylanica} = 0.111$ and $P_{P.\ gardneri} = 0.133$). However, total germination of *P. nigra* in the GA_3 treatment was significantly different from the control ($P_{P.\ nigra} = 0.003$).

trend prior to radicle and plumule emergence (Table 2). Cotyledons developed within the seed, using all the space inside the seed that previously was occupied by the endosperm (Fig. 5).

Discussion

Germination tests conducted in the light at 25°C revealed that *P. zeylanica* seeds first showed germination within 1 month, while those of *P. nigra* took a little over 1 month and those of *P. gardneri* took about 2 months to show first germination. Furthermore, all the viable seeds of *P. zeylanica* and *P. nigra* completed germination within 3 months. However, seeds of the three studied *Psychotria* spp. took >30 d to reach 50% germination (GT_{50} based on the viable seeds), indicating that they had some kind of dormancy. Since the final increase in mass of non-scarified and manually scarified seeds of *P. gardneri* and of *P. zeylanica* did not differ significantly, we concluded that seeds of these species are not physically dormant. Seeds of the three *Psychotria* species have a small embryo with a prominent endosperm, and the embryo grew within the seed prior to radicle emergence. The embryo length:seed length ratio of *P. zeylanica, P. nigra* and *P. gardneri* increased 36, 40 and 57%, respectively, by the time of radicle emergence. Thus, seeds of the three species have a morphological component to their dormancy. Generally, seeds with morphological dormancy (MD) tend to germinate within 1 month. Since the GT_{50} of seeds of the three *Psychotria* spp. was >30 d, these seeds also have physiological dormancy (PD). Therefore, seeds of these three species have a combination of MD and PD, i.e. morphophysiological dormancy (MPD; Baskin and Baskin, 2004). Further, since embryo growth within the seeds of the studied species occurred under warm conditions, seeds have one of the simple levels of MPD. In seeds with one of the complex levels of MPD, embryos required low

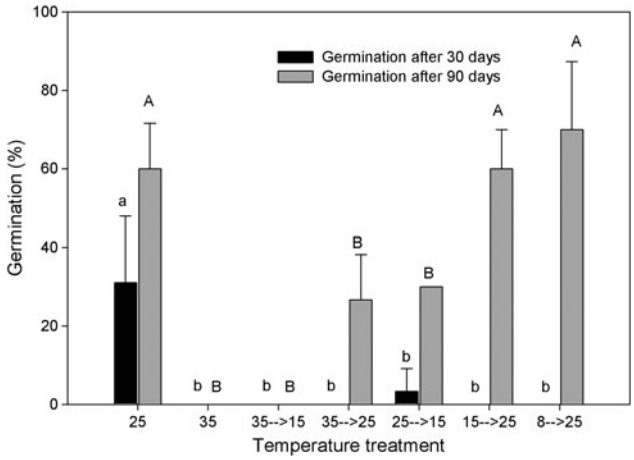

Figure 3. Germination percentages of *P. zeylanica* seeds under different temperature treatments after 30 and 90 d. Germination was grouped by one-way ANOVA using the Tukey method, at 95% CI under Tukey's family error rate of 15. Error bars are + SD.

(0–10°C) temperatures for growth (Baskin and Baskin, 2014).

PD has been classified into three levels: non-deep, intermediate and deep (Baskin and Baskin, 2004, 2014). Non-deep PD can be alleviated by GA_3, while intermediate PD may or may not be broken by GA_3 and deep PD is not broken by GA_3 (Nikolaeva, 1977). Our experiments revealed that 100 ppm GA_3 had no effect on radicle emergence of the three study species (Table 3). All the viable *P. zeylanica* and *P. nigra* seeds had an emerged radicle after incubation for 3 months at 25°C, i.e. ~3 months of warm stratification at 25°C were required to break the physiological component

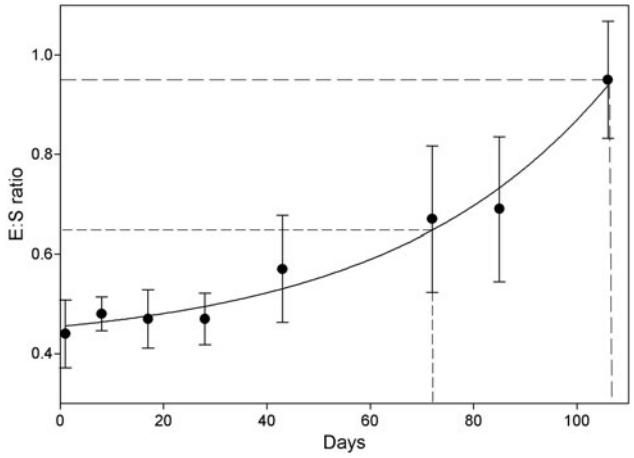

Figure 4. Change in embryo length:seed length (E:S) ratio of *P. gardneri* during the incubation period. The short-dashed line indicates the time (~67 d) of radicle emergence, while the long-dashed line indicates the time (~106 d) of plumule emergence. Stirling's exponential growth curve was fitted to identify the embryo development trend. Error bars are ± SD.

of radicle dormancy in seeds of these two species. This warm stratification requirement is confirmed by the results of the temperature transfer experiments. Seeds transferred from different temperatures took 3 months at 25°C to germinate. Furthermore, in the standard germination test, warm stratification at 25°C for 3 months promoted radicle emergence in seeds of *P. gardneri*. Thus, it can be concluded that the PD in radicles of the study species is non-deep. In the standard germination test, 22.2% of *P. zeylanica* seeds had an emerged radicle within 1 month, indicating that the radicle in this portion of the seeds was non-dormant, while that in the remaining seeds had non-deep PD.

Furthermore, it took more than 30 d for the shoots to emerge from radicle-emerged seeds of *P. zeylanica, P. nigra* and *P. gardneri*, revealing that they have epicotyl MPD (eMPD; Baskin and Baskin, 2014). Remarkably, germinated seeds of *P. zeylanica* remained more than 125 d with a dormant epicotyl in light at 25 and 15°C. Shoot emergence of *P. nigra* seeds with an emerged radicle was first seen within 54 d under standard conditions, while none of the plumules emerged in the GA_3 treatment until 61 d. Thus, plumule emergence of *P. nigra* seeds was not enhanced by the GA_3 treatment. However, epicotyls emerged from seeds of all three species when they were incubated at 25°C for long periods of time (~4 months). Thus, we can conclude that seeds of these *Psychotria* spp. have non-deep simple epicotyl MPD (Baskin and Baskin 2014). The dormancy of the seeds of these species can be described as $C_{1b}B_b$ (radicle)–$C_{1b}B_b$ (epicotyl). C_{1b} means that the root and epicotyl have non-deep PD that is broken by warm stratification, and B_b means that the embryo grows prior to radicle emergence and also prior to epicotyl emergence under warm conditions. This is the first report of epicotyl MPD in the Rubiaceae.

Epicotyl MPD not only occurs in seeds of various temperate species (Baskin and Baskin, 2014), but it has also been reported in a few tropical species, e.g. *Virola koschnyi* (Myristicaceae) and *Minquartia guianensis* (Olacaceae) (Flores *et al.*, 1996). Seeds of some temperate [*Quercus alba, Quercus prinus* (Farmer, 1977)] and some tropical/subtropical species [*Calophyllum brasiliensis, Lecythis ampla* (Flores *et al.*, 1996), *Garcinia kola* (Agyili *et al.*, 2007), *Humboldtia laurifolia, Brownea coccinea* and *Cynometra cauliflora* (Jayasuriya *et al.*, 2012)] with fully developed embryos have been reported to have epicotyl PD (ePD) (Jayasuriya *et al.*, 2012).

Seeds of *P. nigra* dried from 38 to 14% MC germinated to 87%, and those of *P. gardneri* dried from 25 to 12% germinated to 60%. Thus, *P. nigra* and *P. gardneri* seeds have the ability to tolerate desiccation and have either intermediate or orthodox storage behaviour. Since *P. zeylanica* seeds with an initial MC of 15.9% are dispersed during the dry season, it is assumed they have either intermediate or orthodox

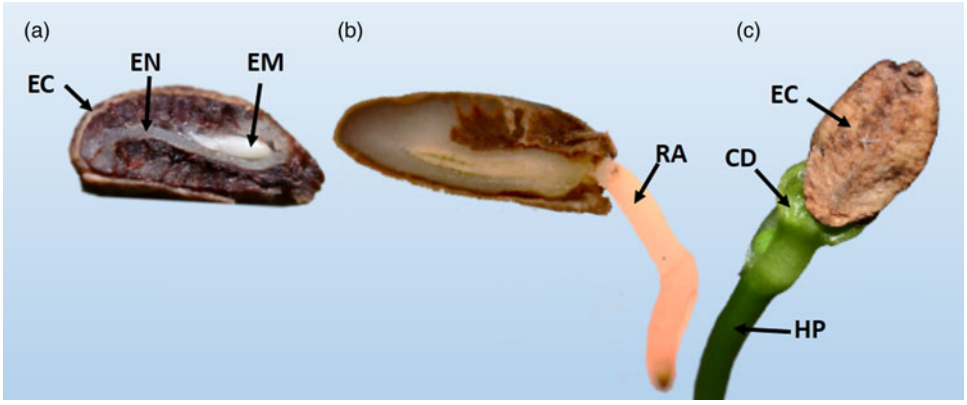

Figure 5. Embryo development in *P. zeylanica* seeds from incubation to complete cotyledon development, i.e. plumule emergence. (a) The small embryo of a fresh intact seed after imbibition in distilled water for 24 h; (b) the embryo after radicle emergence, 34 d after the beginning of incubation; and (c) the fully developed embryo (cotyledons) just before plumule emergence after 125 d. CD, cotyledons; EC, endocarp; EN, endosperm; EM, embryo; HP, hypocotyl; RA, radicle.

storage behaviour, which would help to explain how they remain viable during the dormancy period when exposed to dry habitat conditions.

We recommend incubating montane *Psychotria* seeds at temperatures greater than 15°C and less than 35°C to obtain a maximum number of seedlings from a seed lot. However, even under these optimum conditions, *P. nigra* and *P. zeylanica* seeds took 2 months and 3 months, respectively, for the radicles, and another 2 months for the shoots, to emerge. Further, *P. gardneri* seeds take more than 4 months to produce seedlings. Thus, if seedlings of these three species are needed, it is important to plan the nursery activities properly. These species should be sown about 4–5 months before the time seedlings are required.

Several hypotheses have been proposed to help explain the ecological significance of epicotyl dormancy. Baskin and Baskin (1985) have suggested that epicotyl dormancy may be an adaptation that allows seedlings of species in temperate deciduous forests to have a well-developed root system at the time of cotyledon expansion in early spring. Thus, the shoot has the maximum period of time for growth before canopy closure occurs due to expansion of leaves of the deciduous trees. Furthermore, Jayasuriya *et al.* (2012) have suggested that the presence of epicotyl PD in tropical recalcitrant seeds may be a good adaptation for plants to remain in the understorey until they are exposed to suitable light conditions. Radicle emergence soon after dispersal serves to help maintain viability of the seed via uptake of water. However, seedlings may require a higher light intensity for growth than that occurring under a tropical forest canopy. If plants persist as seedlings with an emerged shoot until a canopy gap is formed, the more palatable parts of the seedling (shoot) could be attacked by pathogens or predators. However, if plants persist as seedlings with the epicotyl dormant, the more palatable shoot parts are protected by the protective layers (seed coats or endocarp) until shoots emerge when they receive suitable light conditions.

Although much more needs to be known to fully understand the significance of epicotyl dormancy in the life history of these species, the available information on two of the *Psychotria* species suggests a possible adaptive mechanism. The peak season of seed dispersal of *P. zeylanica,* which is common in the Horton Plains forests, is from December to February, during which time these montane forests experience dry, low temperature (<15°C) conditions. Temperatures start to increase in mid-February and reach a peak (about 25°C) by March–April; the rainy season begins in April and peaks in July. According to our results, seeds of *P. zeylanica* require about 2 months of warm stratification (at 25 or 15°C) before the radicle emerges. Thus, most of the *P. zeylanica* seeds could germinate (radicle emergence) at the end of March to mid-April, and we have seen seedlings in the field at this time (Athugala, pers. obs.). By the time the rainy season peaks in July, the root system of *P. zeylanica* is well developed, and epicotyl dormancy has been alleviated after 3–4 months of warm stratification. Thus, plumules emerge by July–August when environmental conditions are favourable for rapid seedling establishment and growth. The same adaptive advantage of eMPD can be observed in *P. nigra* seeds in the Knuckles submontane forests. Peak seed dispersal occurs during mid-June to July when the environment is dry, and temperatures are high. A period of about 2 months of warm stratification is required to promote radicle emergence. Thus, the radicles emerge by the end of August to mid-September, at which time the rainy season has started and conditions are favourable for development of a deep root system. The epicotyl in *P. nigra* is dormant and requires about 3 months of warm stratification for dormancy break to occur, resulting in plumule emergence by mid-October to

November at the peak of the rainy season. Thus, dormancy of these two *Psychotria* species seems to be synchronizing radicle emergence to occur at the beginning of the rainy season and plumule emergence to occur at the peak of the rainy season when conditions are most favourable for rapid seedling development.

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Conflicts of interest

None.

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