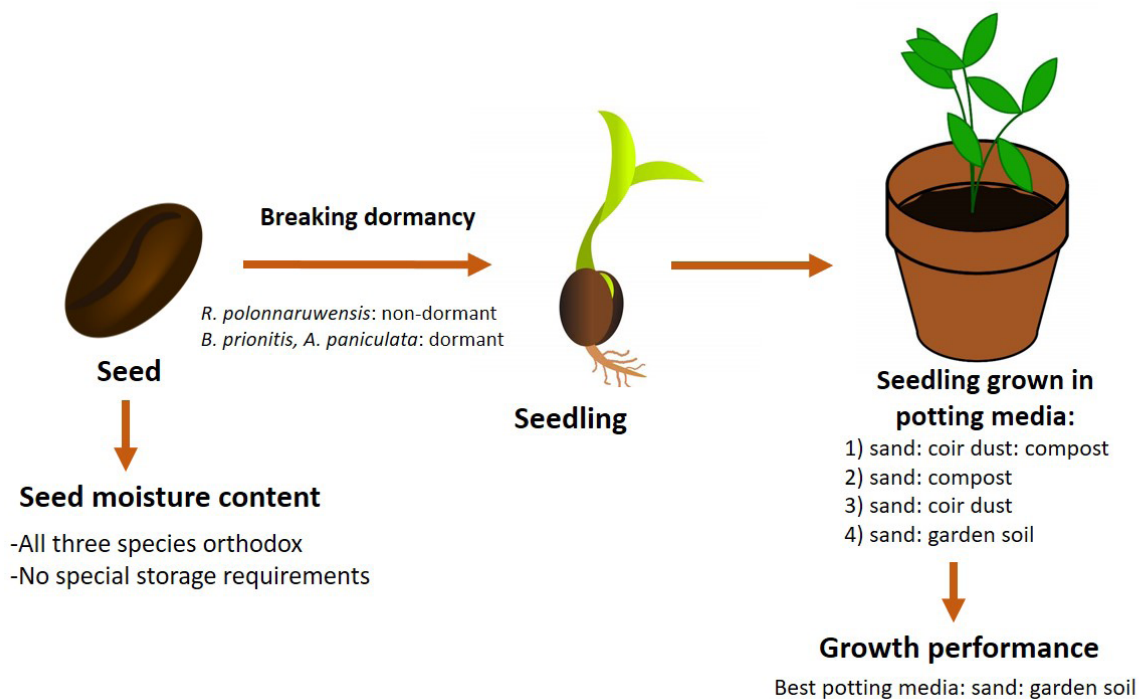


Propagation of three important medicinal plants in Acanthaceae; *Andrographis paniculata*, *Barleria prionitis* and *Rhinacanthus polonnaruwensis*

J. Jayawardhane*, J.W. Damunupola and K.M.G.G. Jayasuriya



Highlights

- Seeds of *Andrographis paniculata*, *Barleria prionitis* and *Rhinacanthus polonnaruwensis* are orthodox and do not need special conditions for long term storage.
- Seeds of *R. polonnaruwensis* do not have dormancy while *B. prionitis* and *A. paniculata* have non-deep physiological dormancy.
- Sand: garden soil potting medium can be recommended as a potential potting medium for these three species.

RESEARCH ARTICLE

Propagation of three important medicinal plants in Acanthaceae; *Andrographis paniculata*, *Barleria prionitis* and *Rhinacanthus polonnaruwensis*

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Abstract: High demand has increased the exploitation of medicinal plants from the wild. Insufficient information on germination and seedling development hinders the propagation of such plants. Thus, seed germination and seedling development of three medicinal species in the family Acanthaceae were studied. Seeds were collected during their peak dispersal period. Seed moisture content (SMC) was determined using the oven dry method. Seed germination and effect of 500 ppm GA treatment and dry storage on germination were studied. Seedlings were transferred to three potting media, viz, sand: coir dust: compost, sand: compost, sand: coir dust and sand: garden soil (in equal ratio) and raised until 45 days in a greenhouse. Number of leaves and height of plants were measured in 7-day intervals. Fresh weight, root, shoot and leaf dry weights were measured after 45 days. SMC of all the studied species was < 15%, revealing that they are orthodox. Thus, no special conditions are needed to store them. *Rhinacanthus polonnaruwensis* seeds had a $T_{50} < 30$ days, indicating that they have no dormancy. *Barleria prionitis* and *Andrographis paniculata* seeds were dormant as the T_{50} was > 30 days. Germination increased > 80 % after GA₃ treatment and dry storage. Therefore, seeds of *B. prionitis* and *A. paniculata* have non-deep physiological dormancy. Dry storage for 1 and 3 months could be recommended for these two species, respectively. Seedlings of all three-study species showed high growth performance in the sand: garden soil potting medium which could be recommended to grow these species. These information will be useful to germinate and grow these three species for large-scale commercial purposes.

Keywords: germination; growth parameters; physiological dormancy; seed dormancy; seedling development.

INTRODUCTION

The demand for medicinal plants has increased throughout the world due to the resurgence of interest in herbal medicines which are known to have a very few or no side effects (Sharma *et al.*, 2006; Sewell and Rafieian-Kopaei, 2014). However, high demand has increased the collection rates of these herbs from the wild populations. Thus, rate of exploitation has increased higher than the rate of natural regeneration (Sharma *et al.*, 2006; Sewell and Rafieian-Kopaei, 2014). Furthermore, the natural habitats of these herbs are depleting at an alarming rate making some of

these species endangered (Sharma *et al.*, 2006; Alves and Rosa, 2007). Therefore, propagation of medicinal plants is of great importance to promote sustainable management, conservation and encourage cultivation of these overexploited species (Nadeem *et al.*, 2000; Sewell and Rafieian-Kopaei, 2014).

Sri Lanka is one of the 34 biodiversity hotspots in the world and comprised of unique assemblages of flora and fauna with high endemism (Gunawardene *et al.*, 2007). However, natural forest cover in Sri Lanka is depleting due to agricultural expansion, land settlements and encroachments and illegal logging. The traditional physicians of Sri Lanka have been using a variety of herbs to prepare various ayurvedic and traditional medicines for different ailments (Ediriweera, 2007). In Sri Lanka, about 150 flowering plant species have been identified as medicinal plants of which several species are endangered (Subasinghe *et al.*, 2003). Therefore, the conservation of these plant species is of great importance (Subasinghe *et al.*, 2003).

Andrographis paniculata var. *paniculata* (Burm. f.) Wall. Ex Nees (create, vernacular name: “green chiretta”), *Barleria prionitis* L. (dog bush, porcupine flower, vernacular name: “Katu karandu”) and *Rhinacanthus polonnaruwensis* L. H. Cramer (vernacular name: “Heen aniththa”) (Table 1) are considered as valuable medicinal plants in Sri Lanka. These three species produce conspicuous flowers (personal observations) and thus could have ornamental values as well. Different parts of *A. paniculata* var. *paniculata* plant contains many secondary metabolites including flavonoids and alkaloids which are responsible for high antimicrobial properties and homeostasis of metabolites under conditions such as hypoglycemic and hypocholesterolemia (Wu, 2008). Thus, different parts of this plant are being used to treat many diseases in dermatology, gastroenterology, and seasonal infections (Joselin and Jeeva, 2014). *B. prionitis* has also being used to treat urinary infections, jaundice, glandular swellings, migraine, oedema, hemoptysis, obesity, cardiac disorder, and Alzheimer’s disease (Ediriweera, 2007; Dheer and Bhatnagar 2010; Gangopadhyay *et al.*, 2012) because it contains a large array of secondary metabolites including

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steroids, tannins and flavonoids with antimicrobial, anthelmintic, anti-diarrheal and cytoprotective activities (Dhawale, 2013). Although information on *R. polonnaruwensis* is not available in the literature, it has also been used as an alternative to *R. nasutus* to treat skin disorders (Wijesooriya *et al.*, 2015). However, large scale cultivation of these species are not available in the country and the materials for medicinal preparations are continuously collected from the wild populations (Personal observations). Thus, the present study emphasizes a preliminary seed germination and propagation protocol for these three species.

Only a few research studies have been conducted to evaluate regeneration and propagation of medicinal plants by using seed-based, clonal and micropropagation techniques (Sharma *et al.*, 2006). Although conventional seeds and stem and root-cutting propagation is a slow process, it is cost-effective. Further, seed-based multiplication is considered the most effective, realistic and convenient method for most plant species (Sharma *et al.*, 2006). Moreover, there is a lack of information on the dormancy, germination, and propagation of these species except for *A. paniculata*. Kumar *et al.*, (2010) has tested the effect of different hormone, hot water and temperature treatments on the germination of *A. paniculata*, speculating physiological dormancy of the seeds (Kumar *et al.* 2011; Baskin and Baskin, 2014). Rawat and Vashistha (2011) also reported that *A. paniculata* could be germinated with GA₃ treatment and high seedling emergence in garden soil covered with dry leaves. Nevertheless, seed dormancy of many Acanthaceae species has been studied and identified as either without dormancy or with physiological dormancy (Baskin and Baskin, 2014).

MATERIALS AND METHODS

Study species

Three important medicinal plant species; *Andrographis paniculata* var *paniculata*, *Baleria prionitis* and *Rhinacanthus polonnaruwensis* were selected for our study. *A. paniculata* var *paniculata* is distributed in India, Sri Lanka, Thailand, Indochina to China. It occurs in semi-shade places, especially in waste grounds. In Sri Lanka, it is distributed in dry and wet low country (Hossain *et al.*, 2014). *B. prionitis* has distributed in tropical Asia and Africa in exposed waste grounds. Normally this plant occurs in the dry zone. *R. polonnaruwensis* is an endemic species which occurs in shady places in secondary semi-evergreen forests of Sri Lanka (Amarasinghe and Wijesundara, 2011). *A. paniculata* was listed as a critically endangered species in the national red data list, while the other two species were listed as least concerned species (MOE, 2012).

Seed collection

Healthy capsules of *A. paniculata* and *B. prionitis* were collected at the dispersal maturity from more than five individuals located at Peradeniya, Sri Lanka, while mature capsules of *R. polonnaruwensis* were collected from Polonnaruwa, Sri Lanka where the species occurs naturally. Capsules were stored in sterilized, brown paper

bags and brought to the Department of Botany, University of Peradeniya and kept until they release seeds within 3 days. Healthy seeds were collected and stored in plastic bottles under ambient laboratory conditions (~ 27 °C and ~ 85% RH). Experiments were initiated within one week of seed collection.

Seed moisture content

Seed moisture content (SMC) indicates seed storage behaviour. According to Hong and Ellis (1996), SMC of orthodox, intermediate, and recalcitrant seeds at maturity was generally < 20 – 50, 23 – 55, and 36 – 90 %, respectively. Ten replicates of ten healthy seeds in each species (100 seeds from each) were weighed separately by using a digital chemical balance to the nearest 0.001 g to determine the initial weight. Seeds were oven dried at 120 °C for 3 h (ISTA, 2018), and reweighed to obtain the dry weight. Seed moisture content of each species was calculated separately using the following formula.

Seed moisture content (Fresh mass basis) = .

$$\frac{(\text{Initial weight} - \text{oven dry weight})}{\text{Initial weight}} \times 100 \%$$

Standard germination

Standard germination test was conducted to determine the seed dormancy. If seeds germinated within 30 days, they are categorized as nondormant seeds, whereas if seeds take > 30 days to germinate, they can be categorized as dormant (Baskin and Baskin, 2004). Two samples containing four replicates each of 25 seeds in each species were incubated on moistened filter papers (with distilled water) in 9-cm-diameter Petri dishes. They were incubated at 25 °C in dark and light/dark conditions in a seed incubator (Model-MGC-250P). Complete darkness was provided by wrapping the Petri dishes with an aluminium foil and the light was provided by cool white fluorescence light. Seeds kept under light/dark cycle were observed for germination in 3-days interval while seeds in complete darkness were observed in 14-days intervals. Observations were continued for 30 days or until all seeds germinated. Radicle protrusion was the criterion for seed germination.

Effect of dormancy breaking treatments on seeds germination

As *A. paniculata* and *B. prionitis* seeds had not germinated within 30 days, seeds were exposed to dormancy breaking treatments to determine the best dormancy breaking treatment as well as the level of seed dormancy. Non-deep level of physiological dormancy (PD) could be alleviated by < 3 months of dry storage or by 500 ppm GA₃ treatment. Intermediate level of PD could be alleviated 3-6 months of dry storage and GA₃ may or may not alleviate this level of PD. GA₃ is not effective in releasing deep level of PD while it requires > 6 months of dry storage to overcome deep PD (Baskin and Baskin, 2014). One sample of four replicates containing 25 seeds of *A. paniculata* was stored dry at ambient laboratory temperature for one month. Retrieved seeds were incubated on filter papers moistened with distilled water in Petri-dishes at ambient laboratory

conditions. Three samples of four replicates containing 12 *B. prionitis* seeds were stored dry at ambient laboratory conditions for 1, 2 and 3 months. Retrieved seed samples were subjected to germination tests as explained above. One sample of four replicates of 15 seeds each from *A. paniculata* and *B. prionitis* were incubated separately on filter papers moistened with 500 ppm GA₃ solution in Petri-dishes at ambient laboratory temperature and light condition. Seeds of all the samples were observed for germination in 3-day intervals for 30 days. Radicle emergence (~ 0.5 mm) was the criterion for germination.

Seedling propagation

After five days, germinated seedlings were transferred into pots (12.5 cm diameter) containing same amount of four different types of potting media (same ratio, either 1:1:1 or 1:1). Four samples of ten seedlings from each species were grown on each media as depicted in table 2. Potting media were selected using readily available materials in the household environment, as one of the important objectives of this study was to determine the propagation method of the three studied medicinal plants, towards promotion of propagation of these species among general public. Soil used in the experiment was similar to the natural habitat of the study species. Soil parameters such as pH, conductivity, texture and colour of each potting medium were recorded before starting the experiment by using digital pH meter (Oakton™ pH-CON 700), conductivity meter (Oakton™ CON 700), feel method and Munsell colour chart, respectively. Healthy seedlings of 3 cm height were used for the potting experiments, which were designed in a completely randomized block in a greenhouse (~ 27 °C and

diffuse sunlight during the daytime and ~ 80% RH). Pots were watered with tap water as necessary. Shoot height, the number of leaves, mortality and survival were recorded weekly for 30 days. Shoot and root fresh weights were determined to the nearest 0.001 g using a digital analytical balance at the end of the experiment. To obtain the dry weight, seedlings were oven dried at 105 °C until they reached a continuous weight.

Data analysis

Data were statistically analyzed using MINITAB 17 (2017) statistical software. One-way ANOVA was conducted, and Tukey's mean separation was proceeded to determine the significant differences in seedling fresh and dry weights of each species in different potting media and germination percentage after different treatments. Germination data were arc-sign transformed prior to conducting one-way ANOVA. Moods median test was used to analyze the differences in the number of leaves of seedlings in different potting media.

RESULTS

Seed moisture content

B. prionitis had the highest dry and fresh mass, while *R. polonnaruwensis* showed the lowest (Table 1). However, the seed moisture contents did not differ from each other and were < 15%.

Standard germination

Approximately, 42% of *A. paniculata* seeds germinated within 30 days at 25 °C in light/dark condition (Figure

Table 1: Fresh and dry mass (g) and moisture content (%) of the mature seeds (mean ± SD, n = 10) of the three tested species

Species	Fresh weight (g)	Dry weight (g)	Seed moisture (%)
<i>A. paniculata</i> var. <i>paniculata</i>	0.049 ± 0.003	0.043 ± 0.002	12.45 ± 2.37
<i>B. prionitis</i>	0.28 ± 0.01	0.25 ± 0.01	11.45 ± 0.43
<i>R. polonnaruwensis</i>	0.0036 ± 0.0002	0.0032 ± 0.0002	11.09 ± 1.78

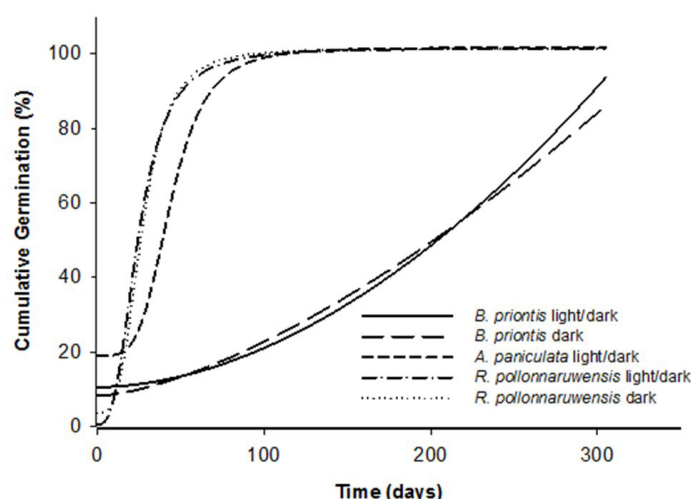


Figure 1: Cumulative germination of *A. paniculata* var. *paniculata*, *B. prionitis* and *R. polonnaruwensis*. Weibull 5 parameter sigmoidal curves fitted to the actual data to model the germination with time.

1), while none of the seeds incubated in dark germinated within 30 days. *A. paniculata* seeds within 70 days at 25 °C in light/dark cycle. Approximately, 18% of the *B. prionitis* seeds kept in light/dark or in complete darkness germinated at 25 °C within 30 days. However, they reached 100% germination after 300 days. In contrast, 100% of the *R. polonnaruwensis* seeds germinated within 30 days at 25 °C in both dark and light/dark conditions.

Effect of different treatments on seeds germination

When *A. paniculata* seeds were stored dry at ambient laboratory temperature for one-month period, germination percentage within 30 days increased from 42% (in the control at 25 °C) to ~ 83% (Figure 2). Further, GA₃ treatment also increased the germination of *A. paniculata* seeds > 85%. However, germination percentage after dry storage on distilled water was not significantly different from that of fresh seeds on 500 ppm GA₃ solution ($F = 10.2$, $P = 0.005$). With the dry storage, germination percentage of *B. prionitis* seeds increased. After 3 months of dry storage at ambient laboratory conditions, they reached 100% germination within 30 days compared to 18% of fresh seeds. Further, fresh *B. prionitis* seeds also germinated to > 95%, when they were incubated on filter papers moistened with 500 ppm GA₃ solution. Germination percentage of *B. prionitis* seeds were significantly higher on filter papers moistened with 500 ppm GA₃ solution and on filter papers moistened with distilled water after 3- month dry storage ($F = 43.8$, $P < 0.001$).

Seedling propagation

Salinity and pH of the potting media

Sand: coir dust: compost potting media and sand: compost potting media showed significantly higher pH than that of sand: coir dust and sand: garden soil potting media (Table 2). Further, sand: garden soil potting medium was slightly acidic (pH 6.69) compared to the rest of the tested media.

Seedling growth in different potting media

Seedling survival

All *A. paniculata* seedlings survived for 30 days (till the end of the experiment) in all potting media tested except in sand: compost medium where 60% mortality was recorded. *B. prionitis* had 10% mortality when grown in sand: compost medium, whereas all individuals survived (100%) in other media tested. In contrast, *R. polonnaruwensis* seedlings showed a 100% of survival only when they were grown in sand: coir dust medium while, 14, 43 and 72 % of the seedlings died in the sand: garden soil, sand: coir dust: compost and sand: compost media, respectively.

Number of leaves

A. paniculata ($\chi^2 = 18.8$, $P < 0.001$) and *R. polonnaruwensis* ($\chi^2 = 10.26$, $P = 0.016$) seedlings grown in sand: garden soil potting media had a significantly higher number of leaves than those in other potting media (Figure 3A). However, a significantly lower number of leaves were observed in *B. prionitis* seedlings grown in the sand: coir dust potting media compared to those in other potting media ($\chi^2 = 9.0$, $P = 0.029$).

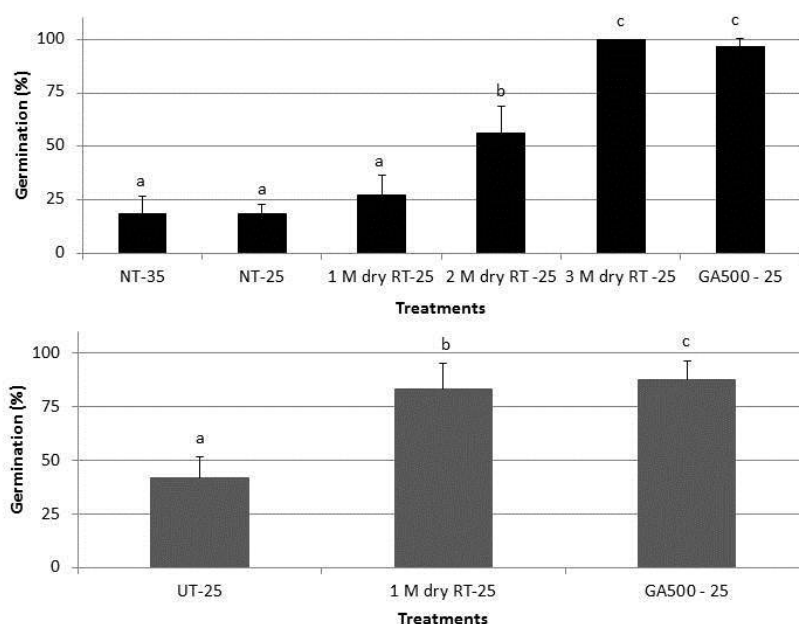


Figure 2: Germination of *A. paniculata* var. *paniculata* (A) and *B. prionitis* (B) seeds after 0, 1, 2 and 3 months of dry storage at ambient laboratory temperature and incubated on filter papers moistened with distilled water or 500 ppm GA₃ solution at 25 or 35 °C temperature in light/dark (12 h/12 h) conditions. Error bars are \pm SD. Different lowercase letters indicate significant differences between treatments within the same species (*A. paniculata* [$F = 10.2$, $P = 0.005$, $n = 4$]; *B. prionitis* [$F = 43.8$, $P < 0.001$, $n = 4$]). NT - 25, non-treated seeds incubated at 25 °C; NT - 35, non-treated seeds incubated at 35 °C; 1 M dry RT - 25, seeds incubated at 25 °C after 1-month dry storage at ambient room temperature; 2 M dry RT - 25, seeds incubated at 25 °C after 2-month dry storage at ambient room temperature; 3 M dry RT - 25, seeds incubated at 25 °C after 3-month dry storage at ambient room temperature; GA 500 - 25, seeds incubated at 25 °C on tissue papers moistened with 500 ppm GA solution.

Table 2: Characteristic features (pH, salinity and colour) of the potting media (ratio either 1:1:1 or 1:1) used during the experiments

Potting media	Sand: coir dust: compost	Sand: compost	Sand: coir dust	Sand: garden soil
pH (at 28.1 °C)	7.79 ^a	7.73 ^a	7.21 ^b	6.69 ^c
Conductivity (at 28.1 °C, dS/m)	3.18 ^a	3.45 ^a	46.56 ^b	79.4 ^c
Colour	Black-very dark brown	Brown-black	Brown	Dark brown
Texture	Sandy-clay-loam	Sandy-loam	Sandy	Sandy-clay

Different lowercase letters indicate significant differences (at $\alpha = 0.05$) between potting media types (pH [F= 233.54, P < 0.001, n = 10], Conductivity [F = 396.8, p < 0.001, n = 10]).

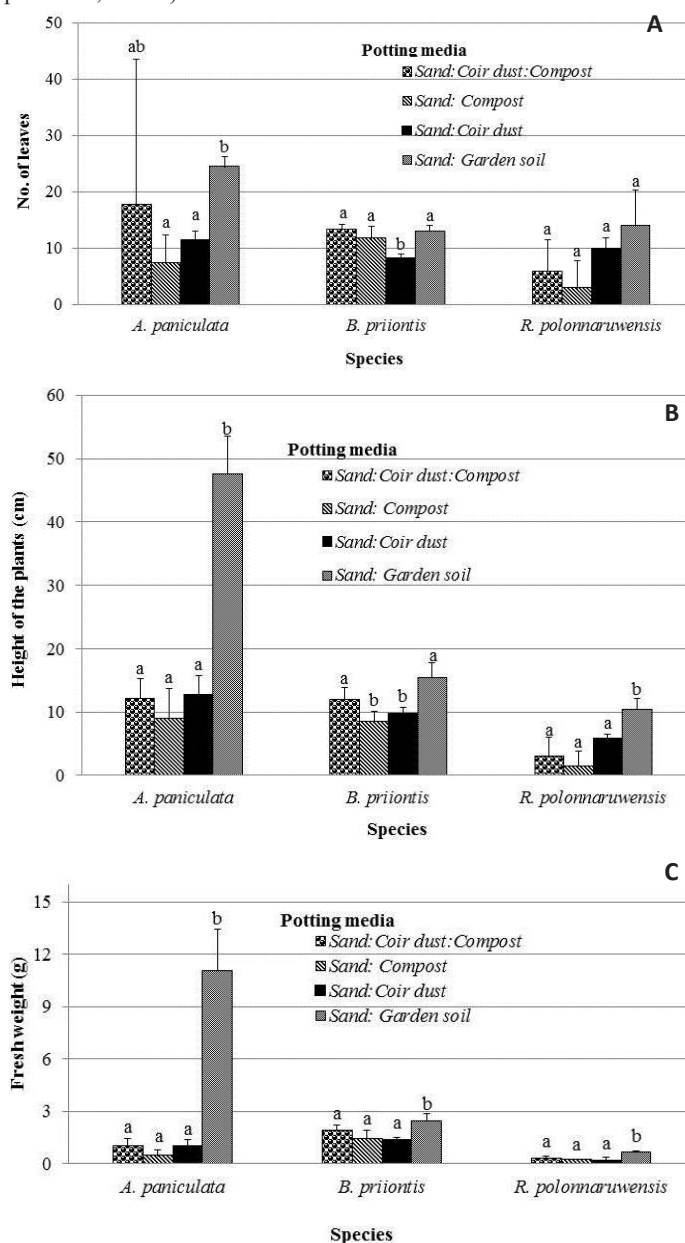


Figure 3: Number of leaves (A), height (B), and fresh weight (C) of *A. paniculata* var. *paniculata*, *B. prionitis* and *R. polonnaruwensis* seedlings grown in different potting media for 30 days. One-way ANOVA and Moods median test, error bars + SD, n = 10. Different lowercase letters indicate significant differences among treatments within the same species (P < 0.001).

Shoot height

The same trend as for number of leaves was observed with plants heights. The tallest *A. paniculata* (F = 103.2, P < 0.001) and *R. polonnaruwensis* (F = 82.7, P < 0.001) seedlings were observed in the sand: garden soil potting

media (Figure 3B). The height of the seedlings in the sand: garden soil medium was significantly different from that of the plants grown in other media. The height of *B. prionitis* seedlings grown in the sand: garden soil and sand: coir dust: compost media are significantly higher than those

grown in the sand: coir dust and sand: compost media ($F = 19.4, P < 0.001$).

Fresh weight

The highest fresh weight of *A. paniculata* ($F = 128.5, P < 0.001$) *B. prionitis* ($F = 10.5, P < 0.001$) and *R. polonnaruwensis* ($F = 12.2, P < 0.001$) seedlings were observed when they were grown on sand: garden soil potting media (Figure 3C). Fresh weight of those seedlings was significantly different from those grown in other potting media.

Root dry weight

A. paniculata ($F = 21.9, P < 0.001$) and *R. polonnaruwensis* ($F = 13.9, P < 0.001$) seedlings grown in the sand: garden soil potting media had significantly higher root dry weight compared to those grown in other potting media (Figure 4A). *B. prionitis* seedlings grown in the sand: garden soil potting media had a significantly higher root dry weight than those grown in sand compost potting media ($F = 3.9, P = 0.015$). However, root dry weight of *B. prionitis* seedlings grown in the sand: garden soil potting media was not significantly different with those grown in other two potting media.

Shoot dry weight

Similar to other growth parameters, shoot dry weight of *A. paniculata* ($F = 88.7, P < 0.001$) and *R. polonnaruwensis* ($F = 24.9, P < 0.001$) seedlings grown in sand: garden soil potting media is significantly higher than those grown in other media (Figure 4B). Shoot dry weight of *B. prionitis* seedlings grown in the sand: garden soil potting media was significantly higher than those grown in the sand: compost or sand: coir dust potting media ($F = 11.26, P < 0.001$). However, it was not significantly different with the shoot dry weight of seedlings grown in the sand: coir dust: compost potting media.

Dry weight of leaves

Same trend was observed in the leaf dry weight of seedlings of *A. paniculata* ($F = 145.7, P < 0.001$) and *R. polonnaruwensis* ($F = 42.4, p < 0.001$). Sand: garden soil potting media gave the highest value which is significantly different from those grown in other potting media (Figure 4C). The highest leaf dry weight of *B. prionitis* seedlings was recorded in those grown in the sand: garden soil potting media which is not significantly different from those grown in the sand: coir dust: compost potting media,

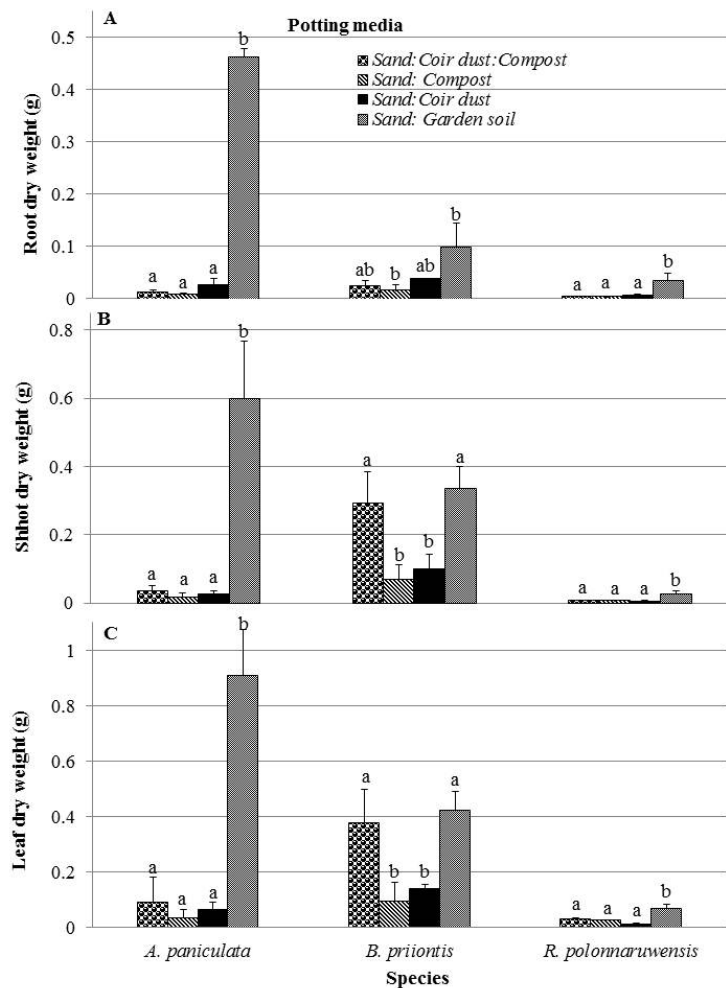


Figure 4: Root (A), shoot (B), and leaves (C) dry weight of *A. paniculata* var. *paniculata*, *B. prionitis* and *R. polonnaruwensis* seedlings grown in different potting media for 30 days. One-way ANOVA, error bars + SD, n = 10. Different lowercase letters indicate significant differences among treatments within the same species ($P < 0.001$).

however, was significantly different from those grown in other potting media ($F = 9.9$, $P < 0.001$).

DISCUSSION

Moisture content of mature seeds of three study species was $< 15\%$ and is within the moisture content range of seeds with orthodox seed storage behavior (see Hong and Ellis, 1996 and Pritchard *et al.*, 2004 for details about moisture range). Since the seeds of the three species reached 100% germination at least after dormancy breaking treatment, it indicates that seeds could survive at this moisture content. Thus, all three seeds could be categorized as orthodox. Orthodox seeds are desiccation tolerant and their storability is comparatively higher than recalcitrant seeds if they are stored under proper conditions (Berjak and Pammenter, 2002; Walter *et al.*, 2013). Seed storage life of orthodox seeds could be increased when they were stored under low temperatures (-5 to 10 °C) (Vertucci and Ross, 2008) at $10 - 12\%$ RH (Hong and Ellis, 1996). Further, even under ambient temperature and humidity conditions seeds of many orthodox species retain viability for a considerable time even without applying a specific storage treatment (Wilson, 1995). As the species is having orthodox seeds, we suggest to store these seeds in sealed polythene bags with 12% seed moisture content.

According to our results, *R. polonnaruwensis* seeds do not have any dormancy. However, *A. paniculata* and *B. prionitis* seeds have dormancy. Seeds of these two species increased their volumes when they were kept on moistened filter papers during the germination test showing signs of imbibition. Thus, these seeds do not contain water impermeable seed coats, i.e., they do not have physical dormancy (see Baskin and Baskin, 2004 and 2014 for information about dormancy classes). Further, the seeds of these two species were totally filled with well-developed cotyledons with the embryo axis, indicating that seeds of these two species have no morphological component to its dormancy. Seeds of both species responded to 500 ppm GA_3 treatment as they germinated $> 90\%$ within 30 days on filter papers moistened with 500 ppm GA_3 . Moreover, *A. paniculata* seeds reached $> 85\%$ germination when they were stored dry for 1 month at ambient laboratory temperature whereas, *B. prionitis* seeds required 3 months dry storage to reach 100% germination. GA_3 and short-term (< 3 months) dry storage alleviate non-deep physiological seed dormancy (Baskin and Baskin, 2004). Thus, according to Baskin and Baskin (2004), it can be concluded that the seeds of these two species have non-deep physiological dormancy. This observation confirms the speculation of Baskin and Baskin (2014) that the *A. paniculata* seeds have physiological dormancy. However, as none of the *A. paniculata* seeds germinated under complete darkness, it indicates light requirement for their germination. Our observations are also in accordance with the research findings on other Acanthaceae species, where other Acanthaceae species were reported with seeds having either no dormancy or physiological dormancy. For propagation purposes, we suggest using non-treated fresh *R. polonnaruwensis*, 1-month-dry stored *A. paniculata* and 3-month-dry stored *B. prionitis* seeds to obtain more

synchronized higher percentage germination.

Seed germination behavior is an adaptation of plants for their respective environments (Donohue *et al.*, 2010). This is also true for our study species. All three study species generally occur in the dry zone of Sri Lanka, although they have been recorded in the wet and intermediate zone sites too. Fruiting season of *R. polonnaruwensis* is in late October or early November (Amarasinghe and Wijesundara, 2011) just prior to the rainy season of the dry and the intermediate zones of Sri Lanka (Herath and Ratnayake, 2004). Thus, they do not require dormancy as they can germinate as soon as they get dispersed along with the favorable conditions for seedling growth during the rainy season. However, both *B. prionitis* and *A. paniculata* produce fruits much earlier i.e., in end of June (Amarasinghe and Wijesundara, 2011) and thus, their seeds needed to stay in the soil until the true rainy season. During this time, intermittent rain could be observed, though may not be favorable for seedling development as most of the period is dry during this time in the dry and intermediate zones. Therefore, seeds of these two species are dormant in order to synchronize germination to the rainy season. Both species require dry storage to alleviate dormancy, which they could get during this period from dispersal to the rainy season. Further, as they have a non-deep level of physiological dormancy. They may also have the ability of cycling of dormancy as many seeds with non-deep level of physiological dormancy could cycle between dormancy and non-dormancy (Baskin and Baskin, 2014). Therefore, seeds of these two species could stay in the soil seed bank for long time periods. However, more experiments are needed to be done to evaluate the presence of dormancy cycling in the seeds of these two species.

When considering seedling survival, sand: coir dust: compost, coir dust: compost and sand: garden soil potting media showed higher seedling survival for *A. paniculata*. However, when other growth parameters (number of leaves, shoot height, fresh weight, and root, shoot and leaves dry mass) were compared, *A. paniculata* plants grew in optimum capacity in sand: garden soil potting media. The number of leaves in *B. prionitis* plants was significantly low when they were grown in sand: coir dust medium. Further, plant height was also significantly low when seedlings were grown in sand: coir dust or sand: compost medium. Although we could not see much difference between plants grown in sand: coir dust: compost medium and sand: garden soil medium, when growth parameters such as number of leaves, height of the plants, shoot dry weight and leaf dry weight are considered, *B. prionitis* plants showed its best performance on sand: garden soil medium as the root dry weight and fresh weight of plants grown in sand: garden soil medium is significantly higher than that of plants grown in sand: coir dust: compost medium. Similarly, when all the above growth parameters are considered, *R. polonnaruwensis* plants showed the highest performance when they were grown in the sand: garden soil potting medium. We speculate that this must be due to favourable pH condition (6.9, acidic), the texture of the soil mix and the nutrient conditions of garden soil. Thus, according to the plant growth experiment, we could recommend that sand: garden soil medium as the best medium to grow the

three studied species.

These three species are usually found in waste grounds or along forest margins (Cramer, 1998). Thus, they may not require soil with high organic matter. Moreover, it seems that all three species required well-drained soil rather than water-retained soil. Soil with high organic matter retains a high amount of water (Gupta and Larson, 1979). Thus, soil with high organic matter content may impede the growth of seedlings of the test species. This may be the reason why the test species have not shown high growth performances in the sand: compost, sand: coir dust and sand: coir dust: compost growth media. In those growth media, coir dust and compost retain water and thus, cause growth retardation of seedlings of the studied species. Furthermore, coir dust could have some phenolic compounds, which may inhibit the growth of seedlings of some of the species (Ma and Nichols, 2004). This may be another reason why higher mortality of seedlings of studied species was observed in potting media containing coir dust. Rawat and Vashistha (2011) also showed that a higher amount of *A. paniculata* seedling emergence occurred in garden soil covered with dry leaves. However, they have not monitored seedling development and seedling growth parameters.

CONCLUSIONS

Our study confirms that seeds of all three species are orthodox as they had a seed moisture content below 15%. Therefore, they do not pose special storage requirements. *R. polonnaruwensis* seeds do not have dormancy ($T_{50} < 30$ days) while *B. prionitis* and *A. paniculata* seeds were dormant ($T_{50} > 30$ days). However, application of dormancy breaking treatments (GA_3) and dry storage increased the germination ($> 80\%$) of *B. prionitis* and *A. paniculata* which represents their non-deep physiological dormancy. Thus, dry storage for 1 and 3 months could be recommended to enhance germination of *A. paniculata* and *B. prionitis* seeds during the preparations for nurseries. Seedlings of all the three test species grew well in the sand: garden soil potting media and thus, it could be recommended for growing these three species. The germination and seedling propagation information could be used to popularize the cultivation of these valuable medicinal plants in large scale for commercial purposes or even at home gardens as ornamental plants. This will also help reducing the rate of exploitation of these species in the wild.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

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