Effect of scarification and soaking on seed germination and viability of *Podocarpus henkelii* and *P. latifolius* (Podocarpaceae)

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Short Report

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Abstract

Seed dormancy defines the environmental conditions in which a seed can germinate. Germination is timed to avoid unfavourable environmental conditions for plant establishment and growth. Podocarpaceae have an underdeveloped embryo which may delay germination. We aimed to establish if *Podocarpus henkelii* and *P. latifolius* produce underdeveloped embryos and examine the kind of dormancy the seeds have. The seed characteristics of *P. henkelii* and *P. latifolius* were observed, and we tested for seed germination after pretreating the seeds in water, hydrochloric acid and mechanical scarification and then tested for viability and the presence of the embryo. Embryos in fresh seeds of *P. henkelii* and *P. latifolius* had an embryo length/seed length ratio < 0.5 which suggested that the embryos were small and underdeveloped. Both podocarps produced fleshy seeds, *P. latifolius* had the smallest seed (mass: 0.19 g; length: 10.54 mm) and *P. henkelii* the largest (mass: 2.44 g; length: 23.69 mm). After exposing the seeds to the pretreatments, the seeds of both species did not germinate after ~6 months. However, viability testing showed that $>90\%$ of the seeds were viable with $>95\%$ of the seeds having embryos. Considering that the pretreatments did not facilitate seed germination in both species and that both podocarps have underdeveloped embryos that were viable *P. henkelii* and *P. latifolius* may have morphological or morphophysiological dormancy.

Key Messages

Seed dormancy in *P. henkelii* and *P. latifolius* cannot be broken by scarification or soaking if the embryo is underdeveloped, as such their seeds show physiological and morphophysiological dormancy.

Introduction

Seed dormancy is the inability of a seed to germinate under environmental conditions that are favourable for germination, as such seed dormancy has evolved differently among taxa, allowing germination to occur when dormancy has been broken (Baskin and Baskin 2004; Fenner and Thompson 2005). Hence, a diverse range of dormancy mechanisms have evolved to ensure germination under suitable conditions. The most frequently used method for measuring seed dormancy is through observation of the absence of germination, therefore, seed dormancy can be observed as an all-or-nothing event where seeds can show maximum dormancy (all) and non-dormancy/germination (nothing). Seed characteristics play a vital role in meeting the requirements of seed germinations (Fenner and Thompson 2005). However, there are two important distinctions in seed responses which alter seed dormancy: (1) factors that are related to seasonal changes such as temperature and/or photoperiod which affect the sensitivity to other environmental factors (including light and water availability and relative humidity) and thus alter the level of seed dormancy, and (2) factors that immediately indicate that conditions are favourable for germination which can terminate dormancy and induce germination.

There are five classes of seed dormancy, these include physiological (PD), morphological (MD), morphophysiological (MPD), physical (PY) and combinational (PY + PD) dormancy. In gymnosperms, physiological dormancy is the most dominant. Seeds experience different levels of PD ranging from embryos not growing, through to growing and producing normal seedlings (Baskin and Baskin 2004). Morphologically dormant seeds tend to have underdeveloped embryos but are differentiated, these seeds are not necessarily dormant (physiologically) but require time to grow/mature before they can germinate. Underdeveloped embryos are also evident in MPD but these seeds require dormancy-breaking treatment (e.g. stratification). Mechanical or chemical scarification is used to break PY dormancy as it makes the impermeable seed coat permeable to water; while PY + PD is evident in seeds that are impermeable to water combined with embryo dormancy (Baskin and Baskin 2004).

Most gymnosperms podocarps the female gametophyte is larger than the embryo (Ferrandis et al. 2011) which functions to bear gametes and protects and nourishes the embryo (Maseshwari and Singh 1967). Podocarpaceae (podocarps) are reported to have underdeveloped embryos (MD) (Ferrandis et al. 2011; Chen et al. 2013). Several studies on podocarps have shown that germination time varies depending on species and/or environmental conditions required for seeds to germinate. *Afrocarpus falcatus* seeds required one-month moist storage and three weeks of incubation to reach germination (Negash 1992), while *P. angustifolius* seeds required >60 days to germinate when not soaked in water, and 35 days when soaked (Ferrandis et al. 2011). *Podocarpus imbricatus* and *P. neriifolius* germinated over a period of 16 – 63 days and 20 – 67 days, respectively (Ng 1992). Some seeds take a significantly longer to germinate, for example, *P. nubigena* did not germinate when on the surface in a canopy gap and only germinated to 22% after one year in the understory during a two-year experiment (Figueroa and Lusk 2001). Dodd and van Staden (1981) reported that to reach 68% germination *P. henkelii* seeds required ~160 days.

In this study, we determined the germination response of *Podocarpus henkelii* Stapf ex Dallim. & Jacks. and *P. latifolius* (Thunb.) R.Br. ex Mirb.. The objectives were to: (1) establish if embryo growth occurs in *P. henkelii* and *P. latifolius* seeds prior to germination, (2) measure seed germination, (3) assess if *P. henkelii* and *P. latifolius* seeds produce underdeveloped or fully developed embryos, (4) identify the kind of dormancy in these species and (5) measure seed morphological characteristics of *P. henkelii* and *P. latifolius*.

Methods

Seed collection: The species selected for this study were *P. henkelii* and *P. latifolius*. *Podocarpus henkelii* has a restricted distribution and occurs in the midlands of south-eastern South Africa which experiences summer rainfall and *P. latifolius* has a broad distribution and occurs in highland regions and in coastal temperate latitudes. During June 2019, freshly ripened *P. henkelii* and *P. latifolius* seeds were collected from Karkloof, Umgano and Mbona forest in the KwaZulu-Natal province, South Africa, and were combined to form a unique seed lot. The seeds were put into Ziploc bags, packed into cooler boxes at 0 – 4 °C (Noel and van Staden 1976) and transported to the University of the Witwatersrand.

Seed morphological traits: All seeds were tested for viability by submerging the seeds in tap water for 30 minutes; seeds that sunk were viable and were used for the experiment. To investigate seed morphology and embryo development in podocarp seeds fifty fresh seeds of each species were cut open lengthwise with a razor blade and the seed length and width as well as embryo length and width were measured using a microscope.

Seed pretreatments and germination: Four hundred *P. henkelii* and *P. latifolius* seeds were washed with tap water, pepped dry using a laboratory paper towel and at 1 °C until they were used for the experiment. The seeds were then washed with Sunlight liquid (a green detergent), rinsed four times with tap water to ensure that the detergent was completely removed, surface disinfected in 3.5% (w/v) sodium hypochlorite (NaOCl), and then rinsed four times using distilled water before they were placed in beakers. The seeds were divided into four treatments to break seed dormancy: (1) soaking in distilled water for 12 hr (control), (2) placed in a freezer in distilled water for 12 hr (cold stratification), (3) acid scarification by treating them with hydrochloric acid (HCl) of 50% concentration for 30 minutes and (4) mechanical scarification (zester) and soaked in distilled water for 12 hr. After the seeds were soaked, imbibed seeds were introduced into petri dishes containing autoclaved and solidified agar at 15 g.l⁻¹ (Purified agar, Sigma, South Africa). Seeds were inserted into the agar while leaving the micropyle exposed. Each treatment had 10 replicates with 10 seeds per petri dish, thus there were a total of 100 seeds per treatment for each species. Seeds were allowed to germinate in an incubator (Convion plant growth chamber, model E8, UK) at 30 °C/15 °C (day/night), 12/12 hr (day/night) photoperiod and 50% relative humidity (Noel and van Staden 1976). Germination was recorded after seven days of incubation and then every two days for six months. Germination was defined as the emergence of the radicle from the seed (Likoswe et al. 2008), where the radicle is more than 0.5 cm. The remaining seeds that did not germinate by the end of the experiment were tested for viability using a standard procedure tetrazolium test after exposing the embryo (International Seed Testing Association 2007).

Data analysis

Statistical analyses were conducted using the statistical program R ver. 4.1.1 (R Core Team 2021). Differences in the morphological characteristics of the seeds between species were tested using a student t-test. Since the viability percentages data violated the normality assumptions (Shapiro-Wilk test), even after arcsine transformation was applied, the Aligned Ranked Transformation procedure was used from the *ARTool* (Wobbrock et al. 2011; Elkin et al. 2021) R package. Means were separated using ART-C with a Tukey adjustment. Seed germination was not tested statistically as no seeds germinated during the experiment.

Results And Discussion

Both *P. henkelii* and *P. latifolius* produce fleshy fruit, with *P. henkelii* producing larger seeds than *P. latifolius* (mass: $t = 32.478$, $df = 49.8$, $P < 0.0001$; length: $t = -69.093$, $df = 49$, $P < 0.0001$; width: $t = -66.592$, $df = 49$, $P < 0.001$; Table 1). The fruit types and their sizes can be due to their seed dispersal

mechanisms, as plants with fleshy fruits are associated with animal dispersal (Givnish 1980; Herrera 1989; Leslie et al. 2013; Contreras et al. 2017). *Podocarpus latifolius* produces seeds that have an orange, red and purple receptacle at the base of the seed and are dispersed by birds (Geldenhuys 1993). Interestingly, it is not known what disperses *P. henkelii* seeds, therefore we propose three hypotheses: (1) *P. henkelii* may be dispersed by gravity as the seeds are often seen rolling down slopes where they are abundant and seeds and seedlings are in close proximity with parent plants, (2) similar to *Afrocarpus falcatus*, *P. henkelii* may also be bird, bat, and/or monkey dispersed because they have similar appearances (Geldenhuys 1993; Negash 1995, Negash 2003), (3) seeds of *P. henkelii* may lack a dispersal agent since fleshy fruit structures in podocarps evolved before modern dispersants emerged (Leslie et al. 2017) and (4) *P. henkelii* may have previously been dispersed by elephants which have since disappeared from their forests, however elephant remains have been found in their current distribution (pers. Obs.); this may explain why they have fleshy fruits.

Podocarpus henkelii and *P. latifolius* embryos are linear-shaped and have a suspensor which links the embryo to the micropyle. The embryo length/seed length (E:M) in freshly harvested *P. henkelii* seeds was small (E:M = 0.37 ± 0.01) as was in *P. latifolius* (E:M = 0.37 ± 0.02); however, there was no significant difference in E:M between *P. henkelii* and *P. latifolius* seeds ($t = -0.330$, $df = 92.79$, $P = 0.7423$) (Table 1). An underdeveloped embryo is usually relatively small, has an E:M ratio < 0.5 and the embryo must grow within the seed before the radicle can emerge (Baskin et al. 2006a). We could not measure embryo length before the radicle emerged because none of the seeds germinated during the experiment after soaking in water, acid, and mechanical scarification. After 189 days of germination assay, seeds that failed to germinate showed >90% viability in both species (Table 2). There was no significant difference in the viability of *P. henkelii* and *P. latifolius* seeds ($F_{(1, 45)} = 0.3049$, $P = 0.5836$). In addition, the pretreatments did not have an effect on seed viability ($F_{(1, 45)} = 0.4054$, $P = 0.6692$). Of the 97.67% *P. henkelii* and 93.33% *P. latifolius* seeds that were viable, the majority (97.33% and 98.33% of *P. henkelii* and *P. latifolius* seeds respectively) had differentiated embryos. Podocarps have been shown to have seeds with an underdeveloped embryo which grows to maturation as conditions improve (Baskin and Baskin 2005; Ferrandis et al. 2011; Chen et al. 2013). Underdeveloped embryos are considered as a primitive trait, although it can also be found in advanced angiosperms. This suggests that *P. henkelii* and *P. latifolius* undergo either MD or MPD because they both produce underdeveloped embryos that need time to grow before germination can occur. Finally, it is necessary to provide *P. henkelii* and *P. latifolius* seed embryos time to grow so that germination can occur.

After attempting to germinate *P. henkelii* and *P. latifolius* seeds for six months, no seeds germinated. Laughton (1938), Noel and van Staden (1976) and Dodd and van Staden (1981) germinated *P. henkelii* seeds within two – five months of incubation. Geldenhuys (1993) reported 51% germination when seeds are soaked in hydrochloric acid, 2% germination when the seeds pass through bushpigs intestines and 71% germination when soaked in water. *Podocarpus latifolius* showed 86% germination after three months (Adie and Lawes 2009). Interestingly, Bussmann and Lange (2000) showed that *P. latifolius* seeds did not germinate after three months of germination and suggested that germination was

affected by seed storage time. After following all protocols all seeds did not germinate, as such future studies should germinate seeds soon after collection, remove the sclerotesta, germinate seeds in soil instead of agar and germinate the seeds in greenhouse conditions instead of incubators.

Declarations

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Conflicts of interest/Competing interests

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Availability of data and material

The datasets generated during and/or analysed the current study are available in the Open Science Foundation Repository: https://osf.io/qydeh/?view_only=291314b97452404e907003e5fe909bd8

Code availability

The code generated during the current study is available in the Open Science Foundation Repository: https://osf.io/qydeh/?view_only=291314b97452404e907003e5fe909bd8

Authors' contributions

Thando C. Twala: Conceptualization; Methodology; Formal analysis and investigation; Writing – original draft and preparation; Writing – review and editing; Funding acquisition; Resources

Jolene T. Fisher: Writing – review and editing; Funding acquisition; Resources; Supervision.

Ethics approval

Not applicable

Consent to participate

Granted

Consent for publication

Granted

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Tables

Table 1. The morphological comparison on seeds of *Podocarpus henkelii* and *P. latifolius*. Mean $\pm$ SD (n = 50) seed characteristics followed by different letters which indicate significant differences between *P. henkelii* and *P. latifolius* seeds (student t-test). E:M is equivalent to embryo length/seed length.

Species	Type	E:M	Seed			Embryo	
			Mass (g)	Length (mm)	Width (mm)	Length (mm)	Width (mm)
<i>P. henkelii</i>	Fleshy	0.365 $\pm$ 0.764 ^a	2.443 $\pm$ 0.489 ^a	23.694 $\pm$ 3.182 ^a	19.618 $\pm$ 2.324 ^a	8.661 $\pm$ 2.429 ^a	1.794 $\pm$ 0.379 ^a
<i>P. latifolius</i>	Fleshy	0.372 $\pm$ 0.108 ^a	0.107 – 0.304 ^b	10.537 $\pm$ 1.084 ^b	8.047 $\pm$ 0.859 ^b	3.876 $\pm$ 1.042 ^a	0.690 $\pm$ 0.169 ^b

Table 2. Seed viability and presence of an embryo in *Podocarpus henkelii* and *P. latifolius* seeds pretreated in water (control) and hydrochloric acid (HCl) and mechanically scarified.

Species	Pretreatment	Viability (%)	Embryo presence (%)
<i>P. henkelii</i>	Control	91 $\pm$ 5.47	97 $\pm$ 2.13
	HCl	92 $\pm$ 5.12	96 $\pm$ 3.06
	Mechanical scarification	95 $\pm$ 5.00	99 $\pm$ 1.00
<i>P. latifolius</i>	Control	95 $\pm$ 2.69	99 $\pm$ 1.00
	HCl	91 $\pm$ 5.47	99 $\pm$ 1.00
	Mechanical scarification	94 $\pm$ 4.27	97 $\pm$ 2.13