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Variability in hardseededness and seed coat thickness of three populations of *Desmanthus virgatus* (Fabaceae, Mimosoideae)

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Abstract

Desmanthus virgatus (L.) Willd. has significant fodder potential, but its seeds have high and persistent levels of physical dormancy which interfere with its field establishment. The purpose of this study was to analyse the variability of physical dormancy in three populations of seeds from humid and arid regions of Argentina, grown and collected in different years and locations. The hardseededness and the percentage of seeds with intact lenses after a softening treatment and the seed coat thicknesses were compared. The variability observed in the obtained percentages of hardseededness, intact lenses and seed coat thicknesses was related to the origin and the environment in which these seeds had developed. Although further work is needed, arid environments seem to favour the development of thicker seminal layers, being an important factor that determines the impermeable nature of the seeds in this species. This information is required for future genetic improvement programs and for understanding the evolutionary process of the populations of this species in different environments.

KEYWORDS
hardseededness, lens, physical dormancy, seed coat thickness

1 | INTRODUCTION

Desmanthus virgatus is part of a complex of species that has shown significant potential as fodder for livestock (Burt, 1993; Gardiner, Bielig, Schlink, Coventry, & Waycott, 2004; Jones & Clem, 1997; Zabala, Giavedoni, Tomas, & Budini, 2010). However, this species typically produces seeds with high and persistent levels of impermeability to water (hardseededness), which seriously interferes with field establishment (Hopkinson & English, 2004). Seed coat impermeability, or physical dormancy (PY) (Baskin & Baskin, 2014), is common in legumes and provides a strategy for seeds to spread germination across time to reduce the risk of premature death during unfavourable environmental conditions (Bewley, Bradford, & Hilhorst, 2012). Physical dormancy is caused by the presence of one or more palisade layers of lignified Malpighian cells (macrosclereids) which are tightly packed together and impregnated with water-repellent chemicals (Rolston, 1978). The breaking of PY involves disruption or dislodgement of "water-gap" structures, which renders the seeds/fruits permeable (Gama-Arachchige, Baskin, Geneve, & Baskin, 2013). The water-gap region is a morpho-anatomically specialized area that differs from the rest of the seed or fruit coat (Gama-Arachchige et al., 2013). The location, anatomy, morphology and origin of water gaps can differ between and even within families (Baskin, Baskin, & Li, 2000; Gama-Arachchige et al., 2013; Jayasuriya, Baskin, Geneve, & Baskin, 2009).

The water-gap region in *Desmanthus* sp., as in many legumes, is the lens. Hopkinson and English (2004) investigated the mechanisms and control of PY in the three Australian cultivars of the genus *Desmanthus*. They found that a brief immersion of hard seeds in boiling water consistently softened a high proportion of the seeds of all

three cultivars. This boiling water treatment causes ruptures in the palisade layers at the lens, which turns the seeds irreversibly permeable. Similar effects were reported for *D. illinoensis* (Michaux) MacMillan, where a brief exposure of the seeds to fire resulted in permeable seeds (Olszewski, D'Agostino, Groch, & Vertenten, 2013). Rangel (2005) reported changes in the lens structure and germination of seeds of nine genotypes of the *Desmanthus* complex (including seven species) in response to a variation in oven temperatures from 25 to 120°C. These results also revealed wide variation among these genotypes with respect to lens rupture, germination rates and persistent hard seeds as function of the range of applied temperatures (Rangel, 2005).

The intensity of dormancy within a given species can vary at several levels: among populations, within populations and between seeds collected in different years from the same population (Foley, 2001; Lacerda, Lemos-Filho, Goulart, Ribeiro, & Lovato, 2004; Smýkal, Vernoud, Blair, Soukup, & Thompson, 2014). Frequently, the variation in dormancy is reflected by the appearance of the seeds or dispersal units in terms of colour, size and thickness of the coat (Smýkal et al., 2014). The water-gap region is recognized as playing a major role in maintaining and breaking PY and in ensuring plant survival and fitness via timing of seed germination. Therefore, characterization of the diversity of this structural complex is important (Gama-Arachchige et al., 2013). Moreover, the recognition of this variability is a primary requisite for the development of accurate seed testing evaluation procedures and efficient production, handling and processing practices, as well as for the attenuation of specific seed problems through breeding (Souza & Marcos-Filho, 2001).

The impermeability of dry seeds can be due to structural and chemical features of the seed coats (Smýkal et al., 2014; Tran & Cavanagh, 1984). However, despite the large body of empirical data, surprisingly, little is known about the cause and nature of impermeability in legumes (Morrison, McClay, Porter, & Rish, 1998). Thick seed coats seem to be required for seed hardness (Lush & Evans, 1980; Petrova, 2002; Souza & Marcos-Filho, 2001; Tran & Cavanagh, 1984), but few studies have analysed the relationship between seed coat thickness and seed hardness. The aim of the present study was to compare the hardseededness, the ruptures at the lenses and the seed coat thicknesses of three populations of *D. virgatus* seeds collected in different years and locations from humid and arid regions of Argentina.

2 | MATERIALS AND METHODS

2.1 | Study area and seed material

Seeds were collected from three experimental populations of *Desmanthus virgatus* growing in two study areas: an experimental field of the Facultad de Ciencias Agrarias-Universidad Nacional del Litoral (FCA-UNL) Esperanza, Santa Fe (31°27′S, 60°56′W), and an experimental field in Charata, Chaco (27°13′S; 61°11′W). Charata was chosen as an evaluation site because it is located in an agroecological zone of potential diffusion of this species complex. The climate is warm temperate, with a dry winter season. The mean annual temperature is 21°C, and the annual average precipitation is 946 mm with a notable decrease in monthly averages from May to September. The variability in precipitation between years is very remarkable, ranging from 500 to 1,550 mm (Herrera, 2009). Esperanza is a mixed production area of agriculture and dairy farming, and the forage supply consists mainly of alfalfa. The climate is warm wet with a mean annual temperature of 18°C and an annual average precipitation of 1,046 mm, mainly concentrated in the summer (Bianchi & Cravero, 2010; GeoINTA, 2017).

Each of the experimental plant populations used in this study consisted of 25 plants (N = 75 plants) separated from each other by 30 cm and arranged in a completely random design (total dimension of the assay: 400 × 450 cm). Two of these populations had originated from seeds collected from arid (DV2) and humid regions (DV5) of Argentina, and the other was derived from seeds of the cultivar Marc (DV1) obtained from the Australian Tropical Crops and Forages Collection (Table 1). These populations were chosen for their contrasting agronomic performances (Zabala, Pensiero, Tomas, & Giavedoni, 2008; Zabala et al., 2010).

Seeds for all tests were harvested in January of 2008 and 2009 from the same set of plants cultured in each location and were stored in paper bags at 25°C until use. Each population of seeds was represented by a set of seeds harvested from 20 experimental plants. The mass of freshly collected seeds was determined by weighing three replicates of 100 seeds on a balance.

Desmanthus virgatus is an autogamous species (Luckow, 1993; Hacker and Hanson, 1999; Zabala et al., 2010) flowering begins in early September. Fruits take about 3 months to ripen (from October to December). Table 2 shows monthly values for average temperature and accumulated precipitation registered during the ripening period of the seeds.

2.2 | Hardseededness percentage

The variation in the degree of hardseededness between populations (k = 3), locations (k = 2) and years (k = 2) was determined by conducting a test 3 months after harvest (in March 2009 and 2010). Seeds were previously scarified in boiling distilled water for 0, 6 or 12 s (k = 3), placed in Petri dishes with three layers of filter paper (Whatman No. 1) moistened with distilled water and cultivated in continuous darkness at 25°C. Four replicates of 25 seeds were used for each of the 36 treatments. The number of germinated seeds was counted every 2 days for 1 month after sowing. Only seeds showing a 2 mm radicle length were considered germinated. The viability of nongerminated seeds was determined at the end of the experiment with the tetrazolium test. These seeds were manually scarified with sandpaper and soaked in 1% tetrazolium phosphate-buffered solution for 10 hr at 30°C in darkness. The seeds were considered viable if the embryo was completely stained red and nonviable if unstained. Any seeds that did not germinate but remained viable were considered hard seeds. The final percentage of hard seeds or the per cent hardseededness (PHS) was then calculated.

TABLE 1 Origin of the populations of *Desmanthus virgatus* employed in this study

Populations	Id[a]	Phytogeographic province (District)[c]	Latitude	Longitude	Annual average temperature (°C)[d]	Annual rainfall (mm)[d]
DV1	viNW1[b]	Yungas (piedmont forest)	23°25′	64°08′	22–23	700–800
DV2	viNW2	Western Chaco or dry Chaco	25°04′	64°56′	18–19	400–500
DV5	viNW5	Yungas (piedmont forest)	23°45′	64°43′	21–22	900–1,000

[a]Id in Zabala et al. (2008). [b]cv. Marc, originally collected in Salta province (data extracted from Australian Plant Genetic Resource Information Service). [c]According to Cabrera (1994). [d]According to work by Bianchi and Cravero (2010) and GeoINTA (2017).

TABLE 2 Monthly values for average temperature (°C) and accumulated precipitation (mm) in Charata and Esperanza during the ripening periods (O: October, N: November and D: December of 2007 and 2008) of seeds of *Desmanthus virgathus* harvested on January of 2008 and 2009 respectively

		Monthly average temperature (°C)[a]			Monthly accumulated precipitation (mm)[a]		
Location	Year of harvest	O	N	D	O	N	D
Esperanza	2008	20	21	23	50	15	120
	2009	20	25	26	1	66	42
Charata	2008	25	24	27	36	93	203
	2009	24	25	26	99	65	34

[a]Data provided by the National Institute of Agricultural Technology (INTA), Argentina, and FCA-UNL.

2.3 | Percentage of intact lenses

Three replicates of 50 seeds of each of the three populations (k = 3) from the two locations (k = 2) collected in 2009 were scarified with boiling distilled water for 6 or 12 s (k = 2). The scarified seeds were then dried with tissue paper, and the proportion of seeds with intact (IL) (Figure 1b), swollen (SL) (Figure 1c) and cracked (CL) lenses (Figure 1d) were determined for each treatment using a stereoscope microscope. The percentage of seeds with intact lenses (PIL) was calculated to allow comparison with the obtained PHS. The hydration capacity of each seed group (intact, swollen and fractured lenses) was determined by separately moistening the seeds at room temperature in Petri dishes containing three layers of filter paper moistened with distilled water.

2.4 | Effect of the lens on water imbibition

Seeds of the three populations (k = 3) harvested at both locations (k = 2) in 2009 were immersed for 12 s in boiling water to obtain groups of seeds with intact and fractured lenses for determination of whether the lens is the main site of water entry. These seeds were divided into four experimental groups: (a) seeds with fractured lenses waterproofed with a blocking material, (b) seeds with fractured lenses and a blocking material applied to the opposite side, (c) seeds with intact lenses and waterproofed with blocking material and (d) seeds with intact lenses and blocking material applied to the opposite side (K = 4). The blocking material was a water-repellent nail slick (Noxell Corp., Hunt Valley, MD) that was adapted from the work of Li, Baskin, and Baskin (1999). These treated seeds were then placed in Petri dishes containing three layers of paper moistened with distilled water and left at 25°C for 48 hr. The seeds were then classified as fully embedded or not embedded, and the percentage of hydrated seeds (PHYS) was calculated. Three replicates of 15 seeds were employed for each of the 24 treatments.

2.5 | Seed coat thickness

Seeds of the three populations (k = 3) from two locations (k = 2) collected over 2 years (k = 2) were employed for this assay. These seeds were manually scarified with sandpaper (to keep the lens intact) and soaked in distilled water. Once hydrated, they were cut longitudinally with a scalpel. The obtained sections were dehydrated through a series of ethanol/water solutions, dried at the critical point with carbon dioxide (CO_2) and analysed using a scanning electron microscope (SEM; FEI Quanta 200 field emission scanning electron microscope; Scanning Electron Microscopy Service from the Laboratorio de Microscopía del Instituto de Física Rosario, CONICET, Santa Fe, Argentina). The seed coat of five seeds per sample (N = 60) was analysed. The lengths of macrosclereid cells were obtained for two sectors of each seed coat: behind the lens (L) (Figure 2a-b) and opposite the lens (Op) (Figure 2c-d). Each value corresponds to an average of the measurement obtained from three macrosclereid cells per sector per seed coat.

2.6 | Statistical analyses

The data were analysed using the INFOSTAT statistical package (Di Rienzo et al., 2011). Before analysis, all variables were tested for normal distribution and equal variances, and values were arcsine transformed when required. However, the untransformed means are reported in the tables and figures. The PHS data were subjected to a three-way analysis of variance (ANOVA) to evaluate the combined effects of "populations," "locations," "years" and "scarification treatment" and their interactions. A three-way ANOVA was performed to test the effects of "populations," "locations" and "scarification treatment" and their interactions on the obtained proportions of IL, SL and CL seeds. The PIL and PHS of seeds harvested in 2009 were compared by a two-way ANOVA for each scarification treatment, with "locations" and "population" as predictor factors. The PHYS

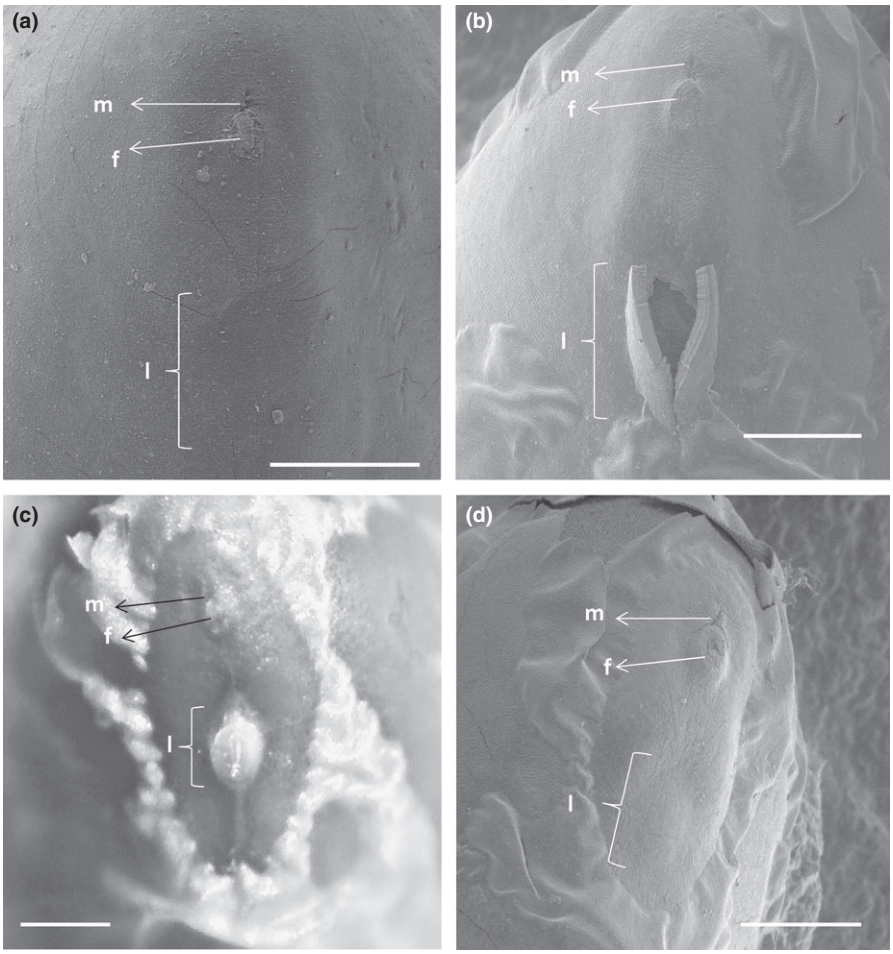

FIGURE 1 Micrographs of the hilar region of *Desmanthus virgatus* seeds showing the micropyle, funiculus and: (a) intact lens of an untreated seed, (b) cracked lens after a brief immersion on boiling water, (c) swollen lens after a similar boiling water treatment and (d) intact lens after a similar boiling water treatment, indicating that this seed has remained dormant. f: funiculus; l: lens; m: micropyle. Bar: 100 μm

variable was analysed by a three-way ANOVA, and the effects of "populations," "locations" and "treatments" and their interactions were assessed. For the "seed coat thickness" experiment, the effects of "populations," "locations" and "years" and their interactions on L and Op were also analysed by a three-way ANOVA. All factors were treated as fixed effects. The differences between mean values were tested for significance using Tukey's test ($p < 0.05$).

3 | RESULTS

3.1 | Hardseededness percentages

Significant effects of the three-way interaction of population*year*locations*treatment were registered for the analysed variable ($F = 7.1$; $p < 0.05$). Hardseededness was then separately analysed for each location and year, with treatment and population as predictors (Figure 3).

The germination of nonscarified seeds was initiated at 3–5 days after sowing and showed the maximum germination percentage after 28 days. These seeds showed a high PHS value (greater than 95%), regardless of the year and location of harvest (Figure 3a-d). Scarification resulted in softening of a high percentage of the seeds. Almost all of these seeds had germinated by 24 hr after sowing.

The germination behaviour of seed populations after scarification treatments was variable. The population of DV2 had the highest percentage of hard seeds for the 2 years and two locations (Figure 3a-d). However, of 30% of PHS registered for the seeds harvested in 2008 (Figure 3a,b), it increased to 50%–60% in seeds harvested in 2009 (Figure 3b,c). In the case of seeds DV5 and DV1, there was an increase in PHS for the seeds harvested in Esperanza 2009 (Figure 3d). From values lower than 20% (Figure 3a–c), values higher than 20% and 40% were observed respectively (Figure 3c).

3.2 | Intact lens percentages

The interaction between locations*population was significant ($F = 70.3$; $p < 0.05$). The seeds of DV2 and E-DV1 had the highest proportion of seeds with intact lens (IL) for the two locations and treatments (Figure 4a,b).

The effects of the scarification treatments were also significant ($F = 40.3$; $p < 0.05$). The treatment consisting of 6 s in boiling water resulted in greater proportions of SL and IL (Figure 4 a) than the proportions registered in the 12-s treatment (Figure 4 b). Unlike the IL seeds, all the seeds with SL and CL were hydrated.

The PIL and PHS (harvested on 2009) obtained after the evaluated treatments were very similar (Table 3). Seeds of population of

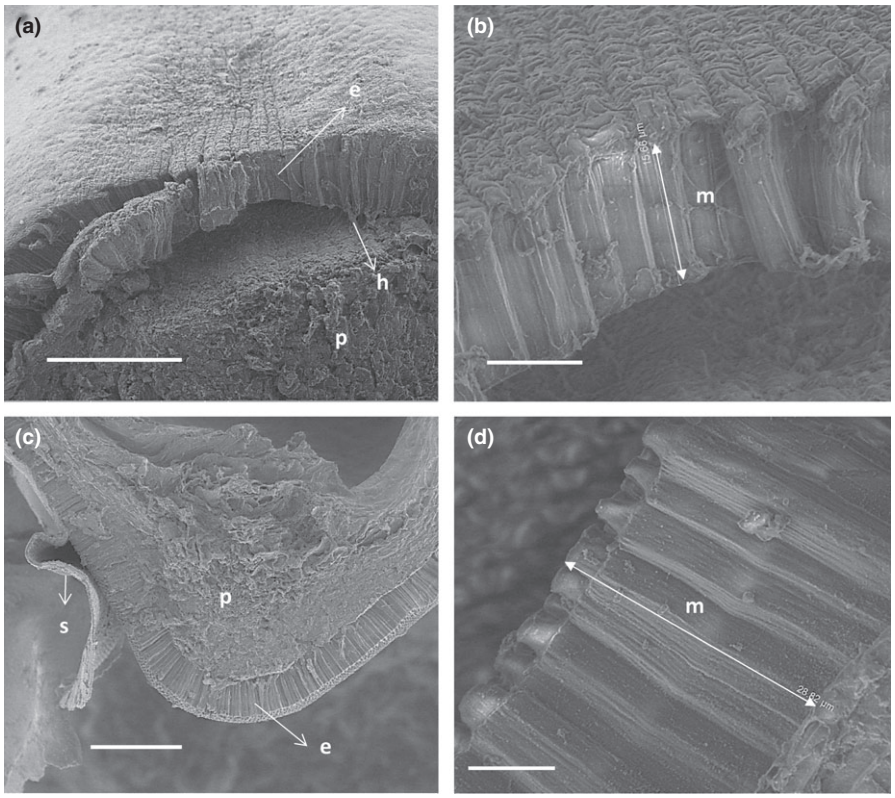

FIGURE 2 Scanning electron micrographs of the seed coats of *Desmanthus virgatus* seed: (a) lens region of the seed showing the epidermis composed of thick-walled macrosclereids arranged in a compact palisade layer, a portion of the hypodermis and the inner parenchyma; (b) length of macrosclereid cells behind the lens; (c) seed region opposite to the lens, showing the epidermis consisting of macrosclereids with a detached scurf layer and the inner parenchyma; and (d) lengths of the macrosclereid cells opposite the lens. e: epidermis; h: hypodermis; m: macrosclereids; p: inner parenchyma; s: scurf. Bar: (a) 50 μm; (b–d) 10 μm; (c) 100 μm

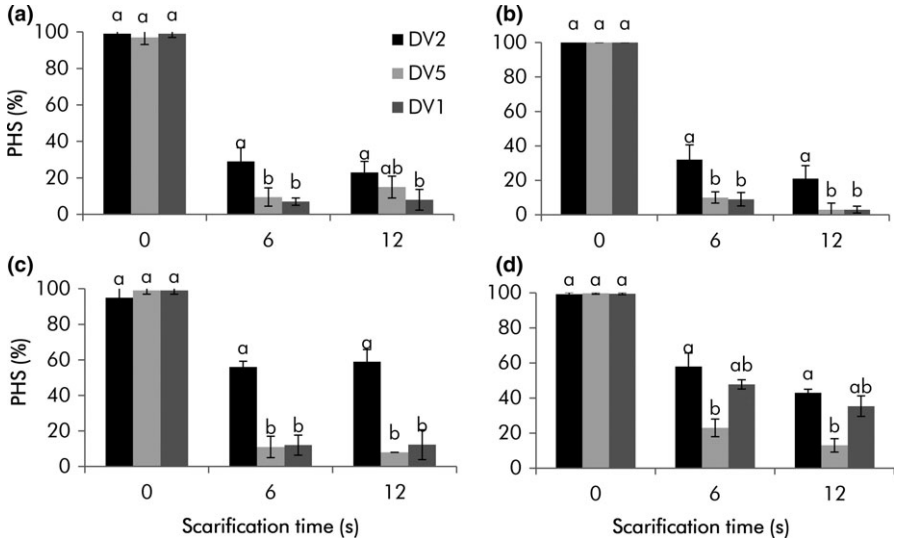

FIGURE 3 Percentage of hard seeds in the three evaluated lines of *Desmanthus virgatus* (DV1, DV2 and DV5) harvested at different locations in different years: (a) Charata, 2008; (b) Esperanza, 2008; (c) Charata, 2009; and (d) Esperanza, 2009. The error bars are *SD*. Means with different letters for each scarification time differ at the 5% level according to Tukey's test

DV2 and the DV1 of Esperanza had the highest PIL and PHS values for both treatments (Table 3).

3.3 | Effect of the lens on water imbibition

The variable PHYS showed a significant effect of the interaction of treatments*populations ($F = 2.3$; $p < 0.05$). The percentages of hydrated seeds between populations were analysed separately for each treatment level.

Blocking the open lenses of scarified seeds with a water-repellent material resulted in approximately less than 50% of the seeds imbibing water in populations of DV1 and DV2, and less than almost 60% in DV5 (Treatment A in Figure 5). In contrast, almost >87% of the scarified seeds with CL became fully imbibed when the opposite

side of seeds was blocked (Treatment B in Figure 5). Conversely, the results for scarified seeds with intact lenses were similar for the three populations regardless of the blocked sector, and some populations reached the 20% of hydrated seeds (Treatment C and D in Figure 5).

3.4 | Seed coat thickness

Significant effects of the three-way interaction population*year*location ($F = 4.2$; $p < 0.05$) were registered for *L* (length of macrosclereid cells behind the lens) (Figure 6). However, only the main effects of populations ($F = 17.8$; $p < 0.05$) and year ($F = 16.5$; $p < 0.05$) showed significant effects on Op (length of macrosclereids at the side opposite to the lens) (Table 4).

These results showed similarities with the data obtained for PHS. The DV2 population had the highest values for seed coat thickness (*L* and Op) (Figure 6 and Table 4 respectively). The populations of DV1 and DV5 had similar values of *L* (Figure 6), but differed in Op (Table 4). A higher Op thickness was obtained for DV1 than for DV5. In addition, the general values of Op were higher for seeds from 2009 (Table 4).

TABLE 3 Percentage of *Desmanthus virgatus* seeds with intact lens (PIL) and percentage of hard seeds (PHS) obtained after two scarification times (6 and 12 s in boiling water) for the three evaluated populations (DV1, DV2 and DV5) harvested from two locations (Charata and Esperanza) during 2009

Scarification time (seconds)	Population	Location	PIL (%)	PHS (%)
6	DV1	Charata	25 ± 1 b	12 ± 6 b
		Esperanza	57 ± 5	48 ± 3 ab
	DV2	Charata	60 ± 6 a	57 ± 3 a
		Esperanza	64 ± 6 a	58 ± 8 a
	DV5	Charata	27 ± 11 b	11 ± 6 b
		Esperanza	31 ± 7 b	23 ± 5 b
12	DV1	Charata	10 ± 9 b	12 ± 8 b
		Esperanza	53 ± 1 a	38 ± 6 ab
	DV2	Charata	49 ± 10 a	59 ± 7 a
		Esperanza	45 ± 9 a	43 ± 2 a
	DV5	Charata	5 ± 2 b	8 ± 1 b
		Esperanza	18 ± 7 b	13 ± 4 b

Note. Means with different letters in each scarification time are different at the 5% level according to Tukey's Test.

4 | DISCUSSION

Untreated seeds of the evaluated populations of *Desmanthus virgatus* did not imbibe water, indicating that these seeds are released from the mother plants with high levels of hardseededness. This constitutes a typical feature of this genus (Hopkinson & English, 2004) and provides them with the possibility of extending seed longevity and persistence in soil seed banks (Gardiner et al., 2004; Hopkinson & English, 2004; Rangel, Henrique, Peter Gardiner, & Lewis Burt, 2015). A brief immersion of the seeds in boiling water can soften a high proportion of the seeds without causing serious damage (Hopkinson & English, 2004); however, a percentage of these seeds will still remain dormant. In the present work, the proportions of seeds that remained dormant were related to the origin of the seeds and

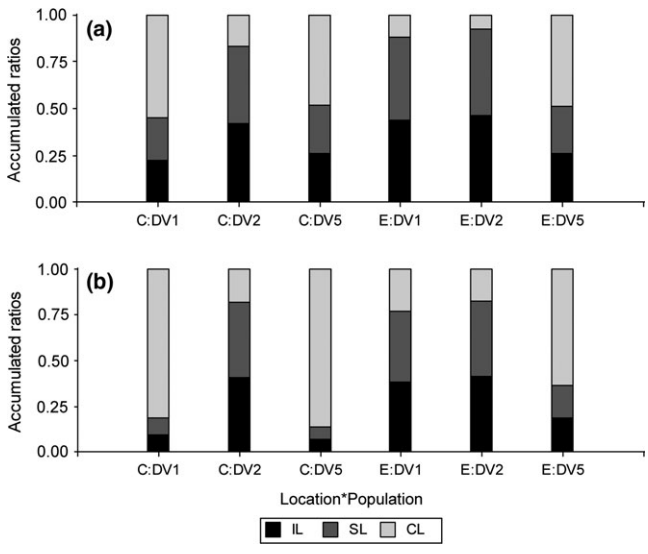

FIGURE 4 Accumulated ratios of the proportion of *Desmanthus virgatus* seeds with intact (IL), swollen (SL) and cracked (CL) lenses after two scarification treatments in the three evaluated populations (DV1, DV2 and DV5) harvested from Charata (C) and Esperanza (E) during 2009: a) 6 s in boiling water and b) 12 s in boiling water

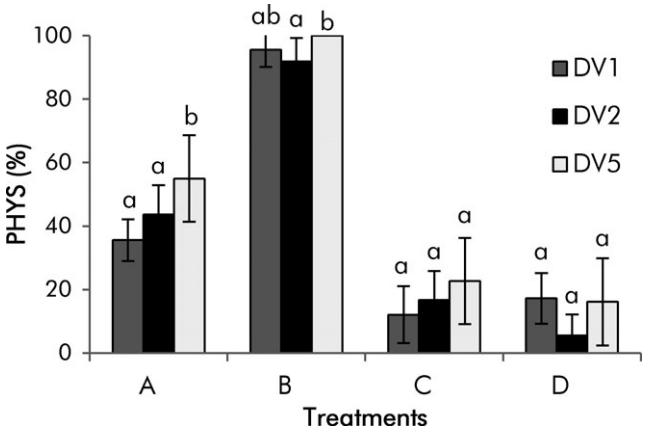

FIGURE 5 Percentage of hydrated seeds (PHYS) of the three populations (DV1, DV2 and DV5) of *Desmanthus virgatus* harvested in 2009, scarified for 12 s in boiling water and subjected to four treatments: (a) seeds with fractured lenses waterproofed with a blocking material, (b) seeds with fractured lenses and blocking material on the side opposite to the lens, (c) seeds with intact lens and waterproofed with blocking material and (d) with intact lens and blocking material on the opposite. The error bars are *SD*. Means with different letters for each scarification time differ at the 5% level according to Tukey's test

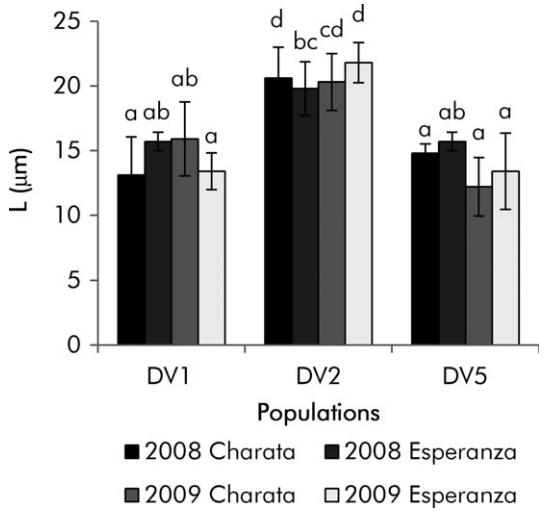

FIGURE 6 Length of macrosclereid cells behind the lens (L) of the three populations (DV1, DV2 and DV5) of *Desmanthus virgatus* harvested in 2009 in different locations and years: Charata, 2008; Esperanza, 2008; Charata, 2009; and Esperanza, 2009. The error bars are *SD*. Means with different letters for each scarification time differ at the 5% level according to Tukey's test

the environment in which these had developed. Variations in the dynamics of seed softening (loss of impermeability) have been described previously in other legumes (Ferreras, Zeballos, & Funes, 2017; Norman, Smith, Nichols, Si, & Galwey, 2006), including nine genotypes of *Desmanthus* (Rangel et al., 2015).

Different requirements for physical dormancy release often arise from the testa structure (Morrison et al., 1998; Serrato-Valenti, De Vries, & Cornara, 1995; Zeng, Cocks, Kailis, & Kuo, 2005). Some evidence supports a relationship between seed coat thickness and physical dormancy release among different legume species (Venier, García, Cabido, & Funes, 2012; Zeng et al., 2005), and among some grain legumes and their wild relatives (Lush & Evans, 1980). Hu, Wu, and Wang (2009) observed differences between the seed coat thicknesses below the hilum in two evaluated populations of *Sophora alopecuroides* L., and they suggested that this difference could partly explain the observed difference in requirements for physical dormancy release. In the present work, we found evidence suggesting a relationship between the percentage of hardseededness after a softening treatment and seed coat thickness among different populations of *D. virgatus*. The population from the arid region showed the highest percentages of PY and the thickest seed coats for the two evaluated locations and years. These results could infer that the impermeable nature of these seeds has a strong component that is associated with the thickness of their seed coats. However, further studies are needed to verify this hypothesis. PY for this species must also have a chemical component, as mentioned for *D. illinoensis* (Olszewski et al., 2013).

The trait of PY is mostly heritable; however, the environmental conditions during seed development play an important role in regulating hardseededness (Jaganathan, 2016), which reflects the fact that the seed coat is of maternal origin (Hudson, Ayre, & Ooi, 2015). Many detailed investigations have confirmed that species with a known history of PY can maintain or lose the dormancy state depending upon the environmental conditions under which the descendent populations are grown (Jaganathan, 2016). Even within the same site, variations in rainfall can produce different proportions of permeable and impermeable seeds within the same plant, in seeds maturing in different years (Jaganathan, 2016; Nichols, Cocks, & Francis, 2009). This work showed that the thickness of the seed coats (in relation to the length of the macrosclereid cells opposite the lens) was higher in seeds harvested in the dryer year 2009 than in the wetter year 2008. Moreover, the percentage of hard seeds was highest in seeds harvested in 2009 in Esperanza, where the amount of rainfall during maturation was lowest. Segura, Vicente, Franco, and Martínez-Sánchez (2015) indicated that mechanically imposed dormancy due to a thick seed coat could be enhanced by drought, which usually increases seed coat thickness and reduces germinability (Baskin & Baskin, 2014; Fenner, 1991). Therefore, maternal environmental factors, such as drought, might influence the degree of dormancy in seeds of *D. virgatus*. It is necessary to develop a more rigorous work in order to solve this hypothesis since, as proposed by Baskin et al. (2000), climatic drying could be an important selective force for the evolution of PY in these species. Identifying controllers of variation in PY is important in the context of changing climate (Hudson et al., 2015).

The anatomy of the seed coat showed similar characteristics to those described for *D. illinoensis* (Olszewski et al., 2013) and some cultivars of *Desmanthus* sp. (Hopkinson & English, 2004). The seed coat of *D. virgatus* is largely covered with scurf, except in the hilar region, and consists of an epidermis, hypodermis and inner parenchyma. The epidermis is composed of thick-walled macrosclereids arranged in a compact palisade layer. The thickness of this layer decreases considerably at the lens, as also reported for *D. illinoensis* (Olszewski et al., 2013) and several species of Mimosoideae and Papilionoideae (Dell, 1980; Rodrigues-Junior, Faria, Vaz, Nakamura, & José, 2014; Serrato-Valenti et al., 1995). This indicates that the lens area is physically the weakest part of the seed coat, and thus, most prone to breakage during different treatments (Baskin et al., 2000; Hu et al., 2009; Rodrigues-Junior et al., 2014; Serrato-Valenti et al., 1995; Ventura de Souza, Voltolini, Santos, & Paulilo, 2012).

TABLE 4 Length of macrosclereids cells opposite the lens (Op) of the three populations (DV1, DV2 and DV5) of *Desmanthus virgatus* and of the 2 years of harvest

	Year		Population		
	2008	2009	DV1	DV2	DV5
Op (µm)	33.0 ± 3.1 a	35.7 ± 3.2 b	33.2 ± 2.8 a	37.1 ± 2.5 b	32.7 ± 3.2 a

Note. Means with different letters in each scarification time are different at the 5% level according to Tukey's Test.

However, in the present work, the thickness of this layer at the lens was very variable and depended on a greater number of variables that was the case for the sector opposite to the lens. This could indicate that the thickness of this layer behind the lens is more sensitive to the maternal environment when compared to its opposite sector, indicating a greater phenotypic plasticity. The confirmation of this, however, will require further studies.

A brief immersion in boiling water causes the buckling of the palisade cells at the lens in all evaluated populations of *D. virgatus* and gives rise to a cleft, as previously mentioned by Hopkinson and English (2004). Similar results were reported following a brief exposure of seeds to heat, which simulated natural events (Olszewski et al., 2013; Rangel, 2005). The blocking experiments in the present work showed that water uptake occurs mainly through the lens. However, a high percentage of the seeds with fractured lenses and waterproofed with a blocking material also underwent hydration. This could reflect an infiltration of water due to the incomplete sealing of the lens and/or the water absorption by other sectors of the seed that could have been damaged during the scarification treatment. No additional sites of water uptake were mentioned in previous work (Olszewski et al., 2013; Rangel, 2005) for this species. However Hopkinson and English (2004) reported that tears could occasionally occur in the testas of immature seeds due to the stresses associated with buckling of the embryo during drying, and these then could become points of first water entry. Hu et al. (2009) reported that seeds may respond differently to different types of dormancy-breaking treatments, which could result in water uptake through different sites (Rodrigues-Junior et al., 2014). These possibilities should be considered in future studies.

In conclusion, the hardseededness in *D. virgatus* could be very variable between populations and over the years due to exposure to maternal environmental factors such as drought during seed development. A large part of this variability would be regulated by the thickness of the seminal covers. Although further confirmation is needed, arid environments seem to favour the development of thicker seminal layers and may therefore constitute an important factor that determines the impermeable nature of the seeds in *D. virgatus*. Additional information in this area will be essential for future genetic improvement programs. This knowledge is also important since it determines the proportion of seeds that become part of the seed bank, thereby modulating the evolutionary process of *D. virgatus* populations in different environments.

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