



Quality of Forage Natural Grass Seeds of the Semi-Arid of Southern Coahuila, Mexico

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ABSTRACT

Semi-arid grasslands in Mexico are characterized by irregular and scarce rainfall, which negatively impacts forage plants and seed quality, which is crucial for the long-term establishment of forage species in pastures. This study aimed to determine the physical and physiological quality of the diaspores and caryopses of eight forage grass species (native and introduced) found in grazing areas in southern Coahuila, Mexico. The caryopses were obtained by manual rubbing using a mat and a rubber pad. Physical quality variables were evaluated, including purity percentage, thousand-seed weight, test weight, and caryopsis morphometry, as well as physiological quality in terms of germination, vigor, and viability. It was found that the introduced species *Sorghum halepense* and *Pennisetum ciliare* exhibited the highest thousand-seed weight values in diaspores, as well as the largest caryopsis size ($p \leq 0.05$). Regarding physiological quality, the native species *Panicum obtusum* recorded the highest germination (80% in caryopses), while *Pennisetum ciliare* showed the highest viability (84%). *S. halepense* exhibited the greatest vigor, with a plumule length of 113.78 mm in diaspores. The native species *Bouteloua barbata* and *Eragrostis lehmanniana* showed zero germination. It is concluded that introduced species stand out for their ability to adapt to harsh environments, which makes them valuable for restoration, conservation, and rangeland rehabilitation schemes. However, native species remain invaluable for maintaining the ecological balance of these areas.

Keywords: arid zones; grasslands; rangeland; small ruminants

INTRODUCTION

Grasslands are a significant source of forage plant diversity. Within these ecosystems, grasses serve as the foundation of livestock nutrition due to their nutritional value, which enhances secondary productivity (Schaub *et al.*, 2020; Blaix *et al.*, 2023). However, extensive grazing in arid zones has low production potential (Quero-Carrillo, 2013). Arid zones are characterized by irregular and scarce precipitation, leading to water-deficient conditions. These conditions, in turn, negatively influence the water relations, physiology, and morphology of forage plants, thereby limiting forage yield and even species survival (Shaaban *et al.*, 2023). In search of alternatives to mitigate these effects, practices are implemented where certain grasses are introduced for forage or erosion control. Nevertheless, many of these are invasive species that disperse throughout ecosystems, replacing native vegetation cover and

resulting in environmental degradation, biodiversity loss, and economic vulnerability (Rai & Singh, 2020; Yang *et al.*, 2025).

Consequently, responsible and efficient management schemes must be developed for the utilization of all resources available in extensive grazing. This is essential to achieve a balance with ecosystem energy flows and ensure high profitability (Quero-Carrillo, 2013). Agroecological practices such as characterizing agroecosystem components, as well as collecting, selecting, and conserving native seeds, can help achieve this (Cartmill *et al.*, 2025). However, prior evaluations are required to consider various physical and physiological aspects of the materials intended for establishment. This approach is highly beneficial for identifying and selecting high-quality seeds that can subsequently be sown with a high success rate to effectively restore and conserve grassland ecosystems (Slodowics *et al.*, 2023). In northern Coahuila, grasslands

dominate the areas where agricultural activities have taken place, but accurate information is needed on the species, their seeds, and their potential for regional management (Contreras-Cisneros *et al.*, 2022; Guillen-Cruz *et al.*, 2022). We hypothesized that, under the semi-arid conditions in southern Coahuila, introduced forage grass species would exhibit seed physical and physiological qualities significantly different from those of native species, and that the removal of accessory structures would increase germination. The novelty of this study lies in the fact that, although livestock farming is a major industry in the region, no studies have been conducted to assess the physical and physiological quality of the forage species found in the grazing lands. Therefore, the objective of this study was to determine the physical and physiological quality of the diaspores and caryopses of eight forage grass species found in grazing areas across four municipalities in southern Coahuila, Mexico, within the region known as La Comarca Lagunera.

MATERIALS AND METHODS

Collection of the evaluated materials was carried out at four sites in La Comarca Lagunera, located in the state of Coahuila. These sites correspond to the municipalities of: Matamoros (25°31'41" N; 103°13'49" W), San Pedro de las Colonias (25°45'28" N; 102°58'59" W), Viesca (25°20'37" N; 102°48'23" W), and Francisco I. Madero (25°46'30" N; 103°16'23" W). The selection criterion for the sites was their location within grazing areas for small ruminants (sheep and goats).

For sampling, field surveys were conducted, and 1,000-meter-long transects were established, where spikes and spikelets of the present forage grass species were 30 randomly collected. Species were identified (Table 1), and diaspores were extracted at the Herbarium and Biotic Resources Laboratory of the Department of Agroecology at UAAAN-UL. Additionally, caryopses were obtained via manual friction using a rubber mat and pad. Subsequently, the samples were stored in paper bags for two to three days at room temperature until analysis.

Several criteria were evaluated to determine physical and physiological quality, in accordance with the methodology described by ISTA (2016) and Ramírez-Segura *et al.* (2022), as described below.

Variable Measured

Purity percentage of the caryopses (% PC). Four random replicates consisting of 10 g of diaspores were taken for each species. From this sample, components were separated into three categories: clean caryopsis, other seeds and inert matter (bracts and soil particles). Individual weights were determined for each category using an analytical balance (Ohaus model AV 114C; capacity: 0.0001-110 g) to calculate the purity percentage and damaged percentage.

Thousand-seed weight (TSW). It was determined using four replicates of 100 diaspores and 100 caryopses for each species. Weight was recorded in grams, multiplying the mean per replicate by ten to determine the final value.

Test weight (TW). This was determined using a 5-mL graduated cylinder, which was filled with diaspores and caryopses until overflowing; the excess was removed by leveling the surface in a zigzag motion. The contents were weighed on an analytical balance (Ohaus model AV 114C; capacity: 0.0001-110 g). Readings were recorded in grams relative to volume (5 mL/g), and data were collected per sample using a single replicate (this variable is presented as descriptive data).

Caryopsis size (CS). All caryopses, as well as the instruments used, were disinfected with 5% commercial sodium hypochlorite, rinsed with distilled water, and immediately sown in Petri dishes. Subsequently, measurements were performed on four replicates of fifty caryopses each to determine the width, length, and thickness for each treatment. The variables considered to evaluate the physiological quality of the seeds were:

Germination percentage (GP). Fifty diaspores and fifty caryopses were sown in Petri dishes on absorbent paper towels, providing the necessary moisture to ensure homogeneous seed imbibition. Subsequently, the Petri dishes were placed in an incubator (Yamato Scientific model IC-403C) at a constant temperature of 25 °C. The count of germinated diaspores and caryopses was conducted over a 12-day period. For the germination curve, only the percentage of seedlings with a well-developed and healthy plumule and radicle was considered.

Table 1. Forage grass species present in the rangelands of the semi-arid region of Southern Coahuila, Mexico

Common name*	Scientific name	Sampling site
Vine mesquite	<i>Panicum obtusum</i> ¹	Francisco I. Madero
Six-weeks threeawn	<i>Chloris virgata</i> ¹	San Pedro, Matamoros, and Viesca
Bermudagrass	<i>Cynodon dactylon</i> ²	Francisco I. Madero, and Viesca
Johnsongrass	<i>Sorghum halepense</i> ²	Francisco I. Madero
Six-weeks grama	<i>Bouteloua barbata</i> ¹	San Pedro, Matamoros, and Viesca
Lehmann lovegrass	<i>Eragrostis lehmanniana</i> ²	San Pedro y Viesca
Buffelgrass	<i>Pennisetum ciliare</i> ²	San Pedro, Francisco I. Madero, and Viesca
Weeping lovegrass	<i>Eragrostis</i> spp.	Francisco I. Madero

Note: *Common names vary by region or municipality; ¹Species native to Mexico and North America; ²Species introduced and naturalized in México.

Vigor (VG). Upon completion of the germination test, the plumule and radicle length were measured in both diaspores and caryopses for each of the four replicates per species. Measurements were taken using a digital Vernier caliper (HER-411 Steren, China).

Viability (PV). Viability was evaluated in twenty-five caryopses, with four replicates per species, using the 0.5% tetrazolium solution (2,3,5-triphenyltetrazolium chloride) imbibition technique. The seeds were first imbibed in distilled water; subsequently, each caryopsis was transversely sectioned to expose the embryonic structures, and only one half of each seed was used for the test. The seeds were then completely covered with the tetrazolium solution. The results were read and interpreted 19 hours later using a stereomicroscope (Olympus-SZX7). The following classifications were considered: Class 1: Fully viable and vigorous embryos, exhibiting uniform pink to bright red staining in the tissues with no visible lesions; Class 2: Viable embryos with medium vigor, showing a lower-intensity pink hue and firm tissues with slight damage, alongside discolored radicles and plumules; and Class 3: Non-viable embryos, appearing colorless, damaged, or

dead (Figure 1). The imbibition time in distilled water was determined for each species, based on a visual assessment of seed swelling. For the purposes of this study, only Class 1 seeds were included in the analysis. Furthermore, imbibition times showed a slight variation of 20 minutes during the evaluation period, ranging between 1 h 10 min and 3 h 15 min.

Statistical Analysis

Statistical analysis was performed (except for the TW variable) using InfoStat software (Di Rienzo, 2020), through an Analysis of Variance (ANOVA) using a completely randomized design with an 8x2 factorial arrangement, where the first factor consisted of eight grass species and the second factor consisted of two seed types (diaspores and caryopses), with 30 replicates per species. Mean comparisons were conducted via Tukey's HSD test ($p \leq 0.05$). The statistical model structure was:

$$Y_{ijk} = \mu + S_i + TS_j + S_i * TS_j + \epsilon_{ijk}$$

Where, Y_{ijk} was a physical or physiological trait; μ was the overall mean; S_i was the i -th effect of the species; TS_j was the j -th seed type effect; $S_i * TS_j$ was the interaction effect species*seed type; and ϵ_{ijk} was experimental error.

RESULTS

The sampling location is situated at an altitude of 1,112 m above sea level (asl) and is characterized by a Bw(h') hw(e) climate. This is defined as an arid-warm climate with an average annual precipitation of 215 mm, a mean annual temperature of 22.7 °C, and an evaporation rate of 2,600 mm (Figure 2).

The results for purity percentage and damaged percentage are presented in Table 2. No interaction effects were found ($p > 0.05$). The highest purity percentage ($p \leq 0.05$) was found in *Eragrostis lehmanniana*, while the species with the lowest filling percentage

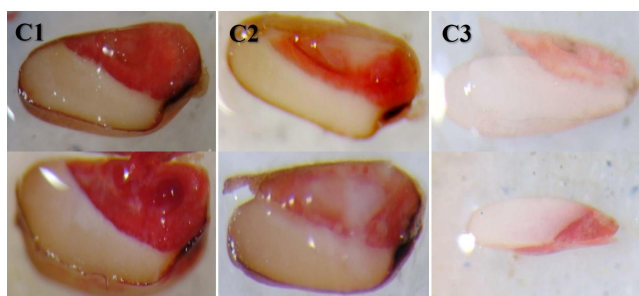


Figure 1. Transverse section of caryopsis. C1) class 1: fully viable and vigorous embryos; C2) class 2: viable embryos with medium vigor; C3) class 3: non-viable embryos. C1 and C2 correspond to *Pennisetum ciliare*, C3 correspond to *Cynodon dactylon*.

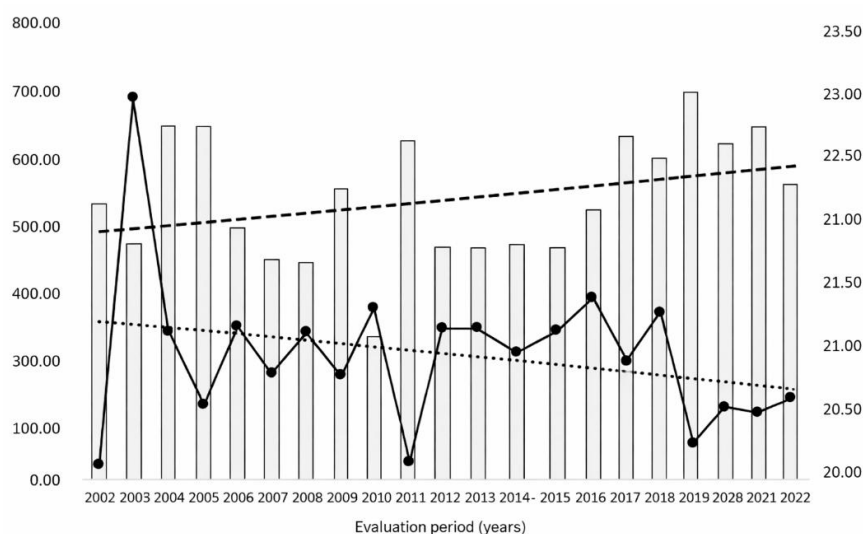


Figure 2. Twenty-year historical records for precipitation (PP) and temperature in the semi-arid region of La Comarca Lagunera, southern Coahuila, Mexico. Note: Average temp. (°C), Precipitation (mm), Trend T°, Trend PP.

($p \leq 0.05$) was *Cynodon dactylon*. Furthermore, the percentage of damaged caryopses showed differences between species: *Panicum obtusum* exhibited the highest percentage of damaged caryopses ($p \leq 0.05$), while *C. dactylon* showed the lowest.

Regarding TSW, *Sorghum halepense* and *Pennisetum ciliare* exhibited the highest weights ($p \leq 0.05$), with 3.95 g and 2.55 g for diaspores and caryopses, respectively. Similarly, the lowest weights ($p \leq 0.05$) were found in *E. lehmanniana* and *Eragrostis* spp., with 0.10 g and 0.08 g for diaspores and caryopses, respectively (Table 2).

The introduced species exhibited the highest TW (Table 3). *E. lehmanniana* showed the highest TW in diaspores (0.97 g), while *S. halepense* recorded the highest TW in caryopses (11.24 g). Regarding caryopsis size, *S. halepense* showed the greatest dimensions in

length, width, and thickness ($p \leq 0.05$). This contrasts with *Eragrostis* spp., which had significantly smaller dimensions, although *E. lehmanniana* showed relatively higher length, width, and thickness ($p \leq 0.05$) within its genus. This is because their size corresponds to the smallest seeds ($p \leq 0.05$) among all evaluated species.

In terms of physiological seed quality, *Panicum obtusum* exhibited the best germination rates for both caryopses and diaspores (Table 4). Conversely, the lowest germination percentages were observed in *E. lehmanniana* and *Eragrostis* spp. Regarding vigor, the species with the greatest plumule and radicle size ($p \leq 0.05$) in both caryopses and diaspores was *S. halepense*. Similarly, the species *Eragrostis* spp. exhibited the lowest vigor in both plumule and radicle for diaspores and caryopses. Finally, viability results

Table 2. Physical quality of forage grasses in rangelands of the semi-arid region of Southern Coahuila, Mexico (mean $\pm$ sd)

Species	Purity Cs (%)	Damaged Cs (%)	TSW (g)	
			Dp	Cs
<i>Pennisetum ciliare</i>	23.11 $\pm$ 2.93 ^{cd}	8.99 $\pm$ 0.93 ^b	3.01 $\pm$ 0.62 ^b	2.55 $\pm$ 0.026 ^a
<i>Sorghum halepense</i>	18.92 $\pm$ 1.85 ^{cd}	6.17 $\pm$ 0.80 ^{bc}	3.95 $\pm$ 0.56 ^a	0.67 $\pm$ 0.02 ^{bc}
<i>Panicum obtusum</i>	18.15 $\pm$ 3.16 ^{cd}	14.43 $\pm$ 2.34 ^a	0.66 $\pm$ 0.06 ^c	0.88 $\pm$ 0.08 ^{bc}
<i>Chloris virgata</i>	25.39 $\pm$ 1.90 ^{bc}	3.58 $\pm$ 0.68 ^c	0.33 $\pm$ 0.02 ^c	0.21 $\pm$ 0.02 ^c
<i>Cynodon dactylon</i>	10.22 $\pm$ 2.09 ^e	3.26 $\pm$ 1.04 ^c	0.23 $\pm$ 0.02 ^c	1.23 $\pm$ 0.16 ^b
<i>Bouteloua barbata</i>	22.68 $\pm$ 1.49 ^{cd}	3.71 $\pm$ 0.73 ^c	0.20 $\pm$ 0.01 ^c	0.14 $\pm$ 0.003 ^c
<i>Eragrostis</i> spp.	30.44 $\pm$ 0.85 ^b	3.88 $\pm$ 0.42 ^c	0.20 $\pm$ 0.01 ^c	0.08 $\pm$ 0.01 ^c
<i>Eragrostis lehmanniana</i>	38.34 $\pm$ 1.87 ^a	6.34 $\pm$ 0.45 ^{bc}	0.10 $\pm$ 0.02 ^c	0.12 $\pm$ 0.01 ^c

Note: Values with different superscript letters in the same column differ significantly ($p \leq 0.05$). TSW: Thousand-seed weight; Dp: Diaspore; Cs: Caryopsis; sd= standard deviation.

Table 3. Test weight for diaspores and caryopses of forage grass species found in rangelands of Southern Coahuila, Mexico (mean $\pm$ sd)

Species	Test weight (g)		Caryopsis size (mm)		
	Dp	Cs	Length	Width	Thickness
<i>Sorghum halepense</i>	0.93	11.24	2.51 $\pm$ 0.21 ^a	1.32 $\pm$ 0.12 ^a	0.79 $\pm$ 0.12 ^a
<i>Pennisetum ciliare</i>	0.46	3.90	1.43 $\pm$ 0.19 ^c	0.79 $\pm$ 0.08 ^c	0.43 $\pm$ 0.08 ^c
<i>Panicum obtusum</i>	0.74	4.17	1.21 $\pm$ 0.11 ^d	0.95 $\pm$ 0.11 ^b	0.60 $\pm$ 0.10 ^b
<i>Chloris virgata</i>	0.72	4.04	1.61 $\pm$ 0.08 ^b	0.38 $\pm$ 0.06 ^e	0.34 $\pm$ 0.06 ^d
<i>Cynodon dactylon</i>	0.94	3.83	1.24 $\pm$ 0.12 ^d	0.56 $\pm$ 0.07 ^d	0.36 $\pm$ 0.06 ^d
<i>Bouteloua barbata</i>	0.49	4.15	0.93 $\pm$ 0.11 ^e	0.40 $\pm$ 0.06 ^e	0.29 $\pm$ 0.05 ^e
<i>Eragrostis</i> spp.	0.66	4.48	0.64 $\pm$ 0.09 ^f	0.31 $\pm$ 0.05 ^f	0.35 $\pm$ 0.05 ^d
<i>Eragrostis lehmanniana</i>	0.97	4.20	0.72 $\pm$ 0.12 ^f	0.32 $\pm$ 0.09 ^f	0.20 $\pm$ 0.03 ^f

Note: Values with different superscript letters in the same column differ significantly ($p \leq 0.05$); Dp= Diaspore; Cs=Caryopsis; sd= standard deviation.

Table 4. Germination, viability, and vigor percentages in forage grass species found in rangelands of Southern Coahuila, Mexico (mean $\pm$ sd)

Species	Germination (%)		Viability (%)	Vigor (length, mm)			
	Dp	Cs	Cs	Plumule		Radicle	
				Dp	Cs	Dp	Cs
<i>Sorghum halepense</i>	3.00 $\pm$ 1.35 ^{cd}	41.00 $\pm$ 1.64 ^b	77.00 $\pm$ 0.18 ^a	113.78 $\pm$ 2.68 ^a	76.68 $\pm$ 2.35 ^a	34.26 $\pm$ 2.20 ^a	31.46 $\pm$ 1.54 ^a
<i>Pennisetum ciliare</i>	12.00 $\pm$ 4.69 ^b	68.00 $\pm$ 3.50 ^a	84.00 $\pm$ 0.15 ^a	33.43 $\pm$ 1.65 ^c	35.19 $\pm$ 0.81 ^b	26.36 $\pm$ 1.31 ^{ab}	25.21 $\pm$ 1.26 ^{ab}
<i>Panicum obtusum</i>	48.00 $\pm$ 11.37 ^a	80.00 $\pm$ 3.17 ^a	75.00 $\pm$ 0.20 ^a	58.30 $\pm$ 1.55 ^b	31.44 $\pm$ 1.18 ^b	22.55 $\pm$ 0.75 ^b	16.03 $\pm$ 0.61 ^{ab}
<i>Chloris virgata</i>	7.00 $\pm$ 1.33 ^{bcd}	33.00 $\pm$ 1.30 ^b	83.00 $\pm$ 0.14 ^a	26.17 $\pm$ 2.94 ^c	26.78 $\pm$ 5.61 ^b	9.69 $\pm$ 1.66 ^c	14.12 $\pm$ 0.44 ^{ab}
<i>Cynodon dactylon</i>	10.00 $\pm$ 0.87 ^{bc}	2.00 $\pm$ 0.74 ^c	53.00 $\pm$ 0.12 ^{bc}	25.36 $\pm$ 1.17 ^c	22.14 $\pm$ 2.04 ^b	10.22 $\pm$ 0.96 ^c	9.63 $\pm$ 1.89 ^b
<i>Bouteloua barbata</i>	0.00 ^d	1.00 ^c	30.00 $\pm$ 0.16 ^d	0.00 ^d	18.76 $\pm$ 5.49 ^b	0.00 ^d	14.70 $\pm$ 7.98 ^{ab}
<i>Eragrostis</i> spp.	0.00 ^d	1.00 ^c	68.00 $\pm$ 0.13 ^{ab}	0.00 ^d	17.78 $\pm$ 1.29 ^b	0.00 ^d	7.87 $\pm$ 2.21 ^b
<i>Eragrostis lehmanniana</i>	0.00 ^d	0.00 ^c	36.00 $\pm$ 0.27 ^{cd}	-	-	-	-

Note: Values with different superscript letters in the same column differ significantly ($p \leq 0.05$); Dp=Diaspore; Cs=Caryopsis; sd= standard deviation.

showed that Buffelgrass (*P. ciliare*) had the highest values ($p \leq 0.05$) for Class I seeds, whereas *Bouteloua barbata* presented the lowest quantity of viable seeds.

DISCUSSION

The results show that the physical and physiological characteristics of the seeds could be influenced by environmental conditions (Scarfó *et al.*, 2024). Regarding the purity percentage of the caryopses, the results could be attributed to the type of spikelets present and the extraction method used to obtain caryopses. In this regard, *Eragrostis lehmanniana* is characterized by having many caryopses per branchlet. Furthermore, regardless of low rainfall conditions, it is able to survive and invade without any adverse effects on its physiological development (Burruss *et al.*, 2022).

For their part, Quigley *et al.* (2023) point out that seed size influences physical quality and is also related to physiological quality. In this particular case, the species *S. halepense* and *P. ciliare* showed a close relationship between TSW and caryopsis size, as they yielded values higher than the rest of the species evaluated. Such data are crucial given that both species are highly invasive and have high dry-matter yields; these characteristics improve their phenotypic plasticity, enabling rapid adaptation to environmental stress (Boyles *et al.*, 2019; Wei *et al.*, 2025). In areas where, for instance, stress conditions—such as high temperatures and low precipitation during seed production—reduce caryopsis size, this could largely explain the observed response in native species (Ravi *et al.*, 2022).

Regarding the physiological quality of the seeds, results found for *P. obtusum* contrast with the report by Anderson and Wright (1974), who state that seed germination for this species is low, resulting in deficient field establishment. Nevertheless, these results open a window of opportunity where it is essential to evaluate the response of each species within its specific agroecosystem. Since germination depends on factors such as genotype, environmental conditions, dormancy, and harvest time, determining optimal germination conditions is necessary to significantly improve the success of field establishment (Lamichhane *et al.*, 2018; Machado *et al.*, 2019). Nevertheless, our study found zero germination in the caryopsis and diaspore stages of *Eragrostis* species. This is likely to explain the low response in our study, as *E. lehmanniana* reportedly needs 6 to 48 months to break dormancy (Wright, 1973), while our seeds were harvested (October/autumn) only four months prior to testing (February/winter).

Other reports (Gutiérrez-Gutiérrez *et al.*, 2022) evaluated germination and vigor using seeds harvested during October with storage periods of two to three years. Their findings showed variations in the days to emergence, with most evaluated species germinating two days after sowing. However, *E. lehmanniana* again showed low germination levels, while *P. ciliare* exhibited the highest values, like our findings.

Seed sizes also appear to influence this attribute, as observed in *S. halepense*. In this regard, studies (Sonkoly *et al.*, 2020) indicate that invasive species possess traits—

such as seed quantity, size, and adaptive germination processes—that contribute to the success of their invasive behavior. This was observed in *S. halepense* and *P. ciliare*, which produce large, heavy seeds that show a marked difference compared to native species and even other introduced species with smaller seeds.

Regarding viability, *P. ciliare* has been reported to enter dormancy and is more tolerant of arid conditions than native species, which could be related to its caryopsis germination percentage (Ravi *et al.*, 2022). Similarly, for this species, the dormancy period has been quantified between 16 and 20 months post-harvest (Stanisavljevic *et al.*, 2020). However, dormancy was not observed in our results, as a high percentage of the seeds were viable. Farrell and Gornish (2019) note that *P. ciliare* modifies water availability by reducing its germination requirements, thus establishing a feedback response for its invasive behavior and allelopathy tolerance. These characteristics could explain its adaptability to the arid and semi-arid conditions of Mexico, as well as its resistance to grazing.

This feedback response of *P. ciliare* triggers a cyclical reduction in native grass populations, which are primarily affected by overgrazing and inappropriate stocking rates. These factors are further intensified by extreme temperature events in arid zones, which undermine their resilience—unlike what occurs with species such as *P. ciliare* (Farrell & Gornish, 2019). This behavior could help explain the low viability found for *Chloris virgata* and *Bouteloua barbata*, a pair of native species that show viability and germination indexes of between 10% and 40%; negative effects are attributed to these factors, compromising their adaptive processes and leading to a diminished presence in rangelands and a greater prevalence of invasive species (Gioria *et al.*, 2026).

This study has some limitations that should be considered when interpreting the results: collection was conducted during a single period, and physiological evaluation was performed after a short storage period; therefore, quality and germination performance may vary across years, seasons, and storage durations.

CONCLUSION

Seed physical and physiological quality differed among the forage grass species evaluated. Invasive species had heavier and larger seeds, which likely contributes to higher germination rates by eliminating bracts and accessory structures. On the other hand, although native species did not show significant results, they persist in the semi-arid region of southern Coahuila. These findings are very important, as they will enable the development of technologies to improve the establishment of forage banks, thereby helping to limit erosion in grazing lands.

CONFLICT OF INTEREST

The Authors declare that there is no conflict of interest.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

The authors declare that no artificial intelligence tools were used in any of the experimental stages, from the conception of the study to the final writing of the results presented in this manuscript.

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