Analysis of dormancy and physiological quality of *Stylosanthes* spp. stored in FGB-UEFS[1]

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ABSTRACT

Knowledge on the viability of the seeds is important in the management of the Germplasm Bank, as it allows monitoring the longevity and the need for regeneration of the accessions. The objective was to validate the efficiency of the Gomez-Campo methodology for seed conservation by evaluating physical dormancy and physiological quality of 11 *Stylosanthes* spp. accessions stored between seven and 13 years in the Forage Germplasm Bank of the State University of Feira de Santana (FGB-UEFS). One-hundred seed were used to determine moisture degree (%) and the viability with the descriptors: percentage of germination (G), germination speed index (GSI), synchronicity (Z) and average germination time (AGT). Sowing was done in plates moistened with water, arranged under controlled conditions (BOD chambers: at 20/30 °C and 12 hours/light) and in a completely randomized design in a 2 x 11 factorial scheme (scarified/non-scarified seeds and 11 accessions). After 14 days, it was observed that most of the accessesion presented high G (greater than 70%), GSI less than 2 seeds.day^{-1}, Z with a mean value of 0,52 and AGT with a mean value of 2 days, characterizing a synchronous and rapid event. Thus, the methodology used in storage is efficient for the conservation of *Stylosanthes* seeds in the medium term.

Keywords: conservation; forage legume; germplasm bank.

INTRODUCTION

Plant Genetic Resources (PGR) represent the basis to assist current and future generations in solving issues related to climate change, loss of biodiversity, environmental degradation and malnutrition (Nass *et al.*, 2012; Bioversity International & CIAT, 2019). Therefore, there is a concern to rescue and conserve germplasm that has potential for use, with emphasis on *ex situ* conservation, with the formation of Germplasm Banks (GBA), which through the storage of seeds enables the conservation of genetics information of the species for a long period of time and in a small physical space (Silva *et al.*, 2018).

As a result, the way of handling the GBA is essential to maintain the longevity of stored seeds even for long periods. Thus, the basic organization of these banks with information such as passport data and characterization of accession; works that follow the viability and vigor in the conservation process in the short, medium and long terms; and suitable environment for storage, are essential (Veiga *et al.*, 2012).

Among the conserved native germplasm, the genus *Stylosanthes* is found. This genus presents a high occurrence and good adaptation to the edaphoclimatic conditions of the Brazilian semiarid region (Oliveira *et al.*, 2016). Works have shown that different species can promote weight gain

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in animals managed on pastures intercropped with grasses, in addition to providing a reduction in the cost of production, thanks to the biological nitrogen fixation capacity of the species (Nagaich *et al.*, 2013; Fabrice *et al.*, 2015).

Despite its economic importance, the germplasm of the species began to be rescued only in 2008 in different mesoregions of the Brazilian semi-arid region and originated the Forage Germplasm Bank of the State University of Feira de Santana (FGB-UEFS) (Oliveira & Queiroz, 2016). Currently, the FGB-UEFS has a collection composed of 370 accessions.

In the FGB-UEFS, the conservation of seed germplasm was adopted, using the methodology of Gómez-Campo (2006), which the author claims to be efficient and of low cost. The initial steps for introducing seeds have not been tested, but since its creation, the material has been conserved *ex situ*, where the seeds are handled and placed in envelopes closed with a galvanized clip, packed in airtight glass containers filled with silica gel for moisture control and kept at room temperature (Oliveira & Queiróz, 2016).

Stylosanthes seeds are classified as orthodox with a desiccation tolerance of 2 to 5% and low temperatures. These species have dormancy that can be integumentary, which can be overcome by high temperatures or mechanical/chemical scarification (Anand *et al.*, 2011) or physiological, which is very pronounced soon after seed maturation, but is gradually lost, until they become completely able to germinate at 12-15 months of post-harvest age (Vieira & Barros, 1994).

Because storage started between seven and 13 years ago, monitoring the viability of the seeds (Araújo *et al.*, 2014) becomes paramount in order to maintain the longevity of the germplasm and/or direct the adoption of regeneration, so that they do not suffer genetic erosion. In this context, the objective of this work was to evaluate the efficiency of the seed conservation methodology recommended by Gómez-Campo (2006) already used in medium term conservation, by observing the physiological quality of the seeds and checking the presence of physical dormancy.

MATERIAL AND METHODS

Site

The experiment was conducted in 2019 at the Forest Garden Experimental Unit of the State University of Feira de Santana-Bahia (UEFS), located at 12º16’00’’ south latitude and 38º58’00’’ west longitude, at 234 m of altitude. The facilities of the Germination Laboratory (LAGER) were used for tests and analyses.

Plant Material

The accessions had been stored since collection, following the methodology of Gómez-Campo (2006), in the Forage Germplasm Bank of the State University of Feira de Santana (FGB-UEFS).

Based on the field book data, 11 accessions were selected considering seed availability and year of insertion in the FGB-UEFS (Table 1). The seeds of the Estilosantes Campo Grande cultivar were purchased from a commercial establishment and had been in storage conditions since 2011.

Seed moisture degree

Moisture content of the seeds was determined using a sample of 100 seeds split into four replications that were weighed on an analytical scale (with four decimal places of precision) to determine the initial wet mass and then taken to dry in an oven of forced air circulation at 105 °C for a period of 24 h (MAPA, 2009). The final dry matter mass was obtained by weighing in an analytical balance. The water content of the seeds was expressed by the moisture degree (MD) considering the initial wet matter (w.m.): MD (w.m.) = ((Wm – Dm) / (Wm-Pr)) * 100, where Wm = Wet matter ; Dm = Dry matter; and Pr = Weight of the container with its lid.

Seed viability of Stylosanthes spp. do FGB-UEFS

The experiment was set in a completely randomized design in a 2 x 11 factorial scheme (scarification and accessions), with four replications of 25 seeds. Seed viability was analyzed using the germination test (Brasil, 2009). For each accession, the material was split into two subsamples composed of 100 seeds each: one with seeds subjected to mechanical scarification, using No. 150 sandpaper for 20 seconds, and the other with seeds with intact seed coat. After the mechanical treatment, they were disinfected in a 0,5% sodium hypochlorite solution for a period of 10 minutes, with subsequent washing in distilled water for the same period.

The seeds were uniformly arranged on a Petri dish containing two sheets of Germitest paper (sterilized in a drying oven at 105 ºC for 4 hours) moistened with distilled water 2,5 times the weight of the paper (MAPA, 2009) and kept in B.O.D.-type germination chambers at temperature ranging from 20 to 30 ºC, with a photoperiod of 12 hours

Table 1: Passport data of the 11 accessions of *Stylosanthes* spp. selected from the FGB-UEFS

Species	Accession[1]	City	Coordinates	Altitude (m)
S. scabra	BGF 008-016	Queimadas	10°54'40"/ 39°12'17"	290
	BGF 014-P110	Ibotirama	12º10'595"/43º14'946"	419
	BGF 014-P130-1	Cristópolis	12º10'558"/44º32'259"	734
	BGF 014-P137-2	Seabra	12º27'311"/42º11'452"	939
S. humilis	BGF 012-014	Canarana	11º48'597"/41º42'066"	809
	BGF 014-P115-2	Muquém do São Francisco	12º13'062"/43º52'018"	836
S. macrocephala	BGF 013-P051-2A	Feira de Santana	12º10'439"/39º01'738"	179
Stylosanthes sp.	BGF 014-P067-1A	Várzea Nova	11º11'942"/40º54'882"	701
	BGF 014-P118-1	Cristópolis	12º33'222"/44º26'433"	718
	BGF 014-P126-2	Luiz Eduardo Magalhães-BR-242	12º06'244"/45º53'530"	784
S. capitata/ S. macrocephala[2]	BGF 011	Feira de Santana	-	-

[1]In all accessions, the three numbers after the letter code (BGF) refer to the year of collection of the seeds and the others represent the passport data identified in the FGB-UEFS. [2]Cultivar Estilosantes Campo Grande: purchased in a shop and maintained in the same storage conditions since 2011.

and remained for a period of 14 days. Those that presented root protrusion between 1 and 2 mm were evaluated daily, considering germinated.

Based on the daily evaluations, the following descriptors were determined: germination percentage (G - in percentage), germination speed index (GSI - seeds/day), germination synchronicity (Z - when at least two seeds germinate together) and average germination time (AGT - days).

Statistical analysis

Data were tested for ANOVA assumptions. Those that did not meet one of the assumptions were transformed and then the univariate analysis of variance was performed. Means were grouped using the Scott Knott test at 5% significance. All analyses were performed using the Genes program (Cruz, 2013).

RESULTS AND DISCUSSION

The seeds stored in the FGB-UEFS showed an average moisture content of 5,21% (Table 2). It was observed that the highest value was observed in *S. macrocephala* (8,01%) and for six of the other evaluated accessions, the moisture contents were less than 5,0%.

This variation in the moisture content found in the seeds stored in the FGB-UEFS is related to the place of conservation, which is considered uncontrolled, with temperature and moisture varying according to the conditions of the environment, where the periodic change of the humidity indicator (silica gel), used in airtight glass containers, constituted the only factor controlling any change in the environmental moisture. Silica gel is a silicon anhydride, SiO_2, with a granular and porous texture, which gives it characteristics that refer to its absorbent property and it acts as a seed conservation mechanism, as it can reduce moisture levels when storage is carried out in impermeable containers, absorbing toxic gases produced during seed maturation (Gómez-Campo, 2006).

The air relative humidity and the temperature together with the moisture content of the seeds represent the most important factors that influence the storage capacity and longevity of the seeds (Smaniotto *et al.*, 2014). When storing germplasm in the medium and long term, more specifically in airtight glass containers kept at room temperature, such as the one used to preserve the FGB-UEFS *Stylosanthes* seeds, attention and initial care must be taken during the processing stage and drying, in order to guarantee water content below 8,0%, which are considered ideal for orthodox seeds (Gómez-Campo, 2006; Goldfarb & Queiroga, 2013).

It should be observed that one of the primordial conditions for the success of the storage of seeds that tolerate desiccation refers to the maintenance of free water contents in the tissues, remaining only the one denominated constitutional or structural water. When the moisture content is less than 10,0%, the water in it is linked to the seed compounds through chemical bonds, such as hydrogen bonds, it does not show mobility and is considered structural, therefore, it

Table 2: Moisture degree of the seeds stored from accessions *Stylosanthes* spp. of FGB-UEFS

Species	Accession	MD (%)
S. scabra	BGF 08*-016	6,07
	BGF 014-P110	4,54
	BGF 014-P130-1	4,53
	BGF 014-P137-2	5,49
S. humilis	BGF 012-014	4,73
	BGF 014-P115-2	5,20
S. macrocephala	BGF 013-P051-2A	8,01
Stylosanthes sp	BGF 014-P067-1A	4,08
	BGF 014-P118-1	4,67
	BGF 014-P126-2	5,50
S. capitata/ *S. macrocephala* [1]	BGF 011	4,41
Mean		5,21

[1] Estilosantes Campo Grande cultivar: purchased from a shop and maintained in the same storage conditions since 2011.

makes its removal difficult (Magistrali *et al.*, 2015). Thus, it is possible to maintain seed viability, especially in long-term conservation (Alves *et al.*, 2017).

In addition, the *Stylosanthes* seeds have a more resistant tegument, and this characteristic was possibly decisive for maintaining the moisture of the seeds at levels considered low, despite the storage location as they were collected at the physiological maturity point, corresponding to the maximum seed mass weight and low water content (Carvalho & Nakagawa, 2012). This fact demonstrates that the presence of a more resistant tegument can contribute as a barrier to the entry of water, if the environment is favored.

The evaluation of the seeds viability (Table 3) showed a significant interaction between the accessions and the scarification procedure to overcome dormancy (scarified and non-scarified seeds) for all descriptors.

The coefficient of variation observed (13,05 to 20,41%) in this work is considered acceptable because the *Stylosanthes* germplasm is a wild material, indicating that it did not undergo artificial selection, therefore, it presents great genetic variability between plants within the accessions (Oliveira *et al.*, 2016). There is no specific classification to quantify the experimental precision in the study of wild species, but these results will be of great value for further works that may arise from the use of the material stored in the FGB-UEFS, particular for the identification of superior individuals to be inserted in the future genetic improvement program of the genus for the Brazilian Northeast.

The results of the descriptors germination percentage (G), germination speed index (GSI) and synchronicity (Z) are shown in Table 4. It was observed that for the descriptor G, all accessions, with the exception of the cultivar *Estilosantes Campo Grande*, showed higher values for seeds with scarified seed coat, in which accessions FGB 08-016 and FGB 014-P110 form the group with the highest percentages (> 98,00%) and accessions FGB 012-014 and FGB 014-P067-1A, are grouped with the lowest averages (< 58,00%). Although more detailed works were not directed towards the aging process of seeds, this variability within the genus of *Stylosanthes* was already expected, as they are wild materials.

Although the germplasm had already been stored for seven and 13 years, approximately, when analyzing the non-scarified seeds, it was observed that there was no expressive overcoming of physical dormancy over this

Table 3: Summary of analysis of variance (ANOVA) for the descriptors evaluated in 11 accessions of the genus *Stylosanthes* spp. subjected to different treatments

Source of Variation	DF[1]	G[2]	GSI[3]	Z[4]
Accessions	10	70,92**	2,44**	0,01**
Treatment	1	42318,32**	122,25**	0,009*
Accessions X Treatment	10	817,59**	2,39**	0,01**
Residue	66	43,61	0,14	0,001
CV[5] (%)		13,63	13,05	20,41
Mean		56,00	8,43	0,52
W		**0,911**	**0,905**	**0,980**
F		**5,801**	**7,173**	**4,706**

[1]DF: Degrees of Freedom; [2]G: Germination (%); [3]GSI: Germination Speed Index (seed/day); [4]Z: Synchronicity; [5]CV: Coefficient of Variation; **,*,ns: significant (p-value < 0,01), (p-value < 0,05) and not significant by F-test, respectively. W; F: statistics from Shapiro Wilk and Levene tests, respectively; values in bold indicate residue with normal distribution and variance at 0,01 significance.

Table 4: Average data of germination and synchronicity of 11 *Stylosanthes* spp. accessions in different environments

ACESSIONS	S[1]	NS[2]	S	NS	S	NS
	G[3] (%)		GSI[4] (seeds day^{-1})		Z[5]	
BGF 08-016	98,60 Aa	7,40 Bd	22,35 Aa	0,81 Bd	0,77 Aa	0,31 Bb
BGF 012-014	54,10 Ad	21,40 Bd	10,77 Ac	3,68 Bc	0,46 Ab	0,46 Ab
BGF 013-P051-2A	88,00 Ab	11,80 Bd	17,85 Ab	1,67 Bd	0,45 Ab	0,38 Ab
BGF 014-P067-1A	57,10Ad	12,60 Bd	6,98 Ad	1,12 Bd	0,35 Ab	0,28 Ab
BGF 014-P110	100,00 Aa	11,80 Bd	23,89 Aa	2,21 Bc	0,84 Aa	0,38 Bb
BGF 014-P115-2	96,50 Ab	52,10 Bb	21,34 Aa	9,73 Ba	0,70 Aa	0,47 Bb
BGF 014-P118-1	76,20 Ac	11,30 Bd	14,01 Ac	1,59 Bd	0,57 Ab	0,49 Ab
BGF 014-P126-2	95,60 Ab	33,70 Bc	16,30 Ab	5,27 Bb	0,44 Ab	0,45 Ab
BGF 014-P130-1	96,50 Ab	8,20 Bd	18,06 Ab	1,21 Bd	0,44 Bb	0,98 Aa
BGF 014-P137-2	91,50 Ac	5,30 Bd	21,60 Aa	1,29 Bd	0,83 Aa	0,98 Aa
BGF 011[6]	82,10 Ac	69,80 Aa	14,76 Ac	11,61 Aa	0,40 Ab	0,32 Ab

[1]S: scarified; [2] NS: without scarification; [3]G: germination; [4]GSI: germination speed index; [5]Z: synchronicity. [6]Estilosantes Campo Grande cultivar: purchased in a shop and kept under the same storage conditions since 2011. Means followed by the same capital letter in the rows and small letters in the columns do not differ from each other, according to the Scott Knot test at 5% significance.

time for most accessions. It is worth mentioning that these non-scarified seeds were kept for more one week (totaling 21 days) in B.O.D. conditions, and even being exposed to ideal germination conditions for a longer period, there was no radicle emission in the seeds that remained in the Petri dishes. From this perspective, the rigid tegument acted as a barrier to root protrusion and technically, mechanical scarification is necessary as attenuator of physical impediment, even after long periods of storage.

Thus, the rigid seed coat was preponderant for maintaining longevity and viability, taking into account that this tegument performs important functions such as protecting the embryo and seed reserves from stress and regulating water absorption during germination (Smýkal *et al.*, 2014). The most resistant tegument is attributed to its composition of macrosclereids, that is, palisade cells, which makes it impermeable to water due to the presence of water-repellent substances such as cutin, lignin, quinones, pectic-insoluble materials, suberin and waxes (Baskin & Baskin, 2014; Smýkal *et al.*, 2014). A similar result was observed by Silveira *et al.* (2014) when evaluating the influence of storage on *Mimosa foliolosa* seeds and note that the seed longevity of this species may be attributed to the hard, water-impermeable seed coat.

However, it should be observed that the performance of the accession FGB 014-P115-2, which presented an expressive result for germination, even in seeds without scarification. *Stylosanthes* seeds, like most legumes, have physiological dormancy that is gradually lost after harvesting and physical dormancy imposed by the seed coat (Chaves *et al.*, 2017). Thus, for this accession, it was observed that in addition to physiological dormancy, there was some percentage of loss of physical dormancy. This can be attributed to the complex interaction between the factors genetic variability, adaptability to the places of occurrence and physiological specificities, and the material used is of wild origin (Salomão *et al.*, 2019).

Ex situ conservation monitoring actions, in the form of seeds, must act in this sense, in recognizing accessions that must be regenerated more rapidly due to low seed viability results obtained during germination tests. In this sense, the BGF-UEFS stood out when to consider that for most of the accessions studied it was found a germination percentage greater than 85,0%, the minimum recommended value so that the seeds can be preserved for long periods (Pádua, 2016).

These results are of great value for the management of FGB-UEFS, as they confirm the viability of the methodology recommended by Gómez-Campo (2006), providing a viable and efficient way of storing germplasm in the short and medium term. In addition, there is a low cost and little demand for infrastructure, compared to other forms of *ex situ* storage, such as seed storage in a cold chamber, cryopreservation, *in vivo* and *in vitro* plant maintenance.

In response to mechanical scarification of the tegument, water enters the internal tissues, which stimulates the growth of the embryonic axis and subsequent root protrusion. As long as the seeds are viable, the speed of root protrusion is influenced, which can be controlled by the number of seeds germinated per day through the germination speed index (GSI). The scarified seeds of all evaluated accessions showed high IVG, with emphasis the grouping on accessions BGF 08-016, BGF 014-P110, BGF 014-P115-2 and BGF 014-P137-2 (Table 4). It is also noteworthy that the referred accessions were those that presented seeds with germination potential greater than 90% and that are seeds stored for a period superior to six years. The high IVG values observed reflect the vigor of the Stylosanthes seeds evaluated in this work (Ranal & Santana, 2006).

The germination synchronization index (Z) is a descriptor that varies between 0 and 1. When the value of Z approaches 1, all seeds germinate at the same time, and when Z approaches 0, at least two seeds can germinate, one at a time (Ranal & Santana, 2006). Accessions BGF 08-016, BGF 014-P110, BGF 014-P115-2 and BGF 014-P137-2, when subjected to mechanical scarification, showed Z values between 0,70 and 0,84, demonstrating uniformity in the germination process, due to the high viability of the seeds. For the non-scarified seeds, it was seen that for most of the accessions the values were close to zero, it can be said that the germination process of the few germinated seeds occurred over time and once again demonstrating that the mechanical scarification was an essential procedure for germination to occur over time.

Despite presenting Z values close to 1, accessions BGF 014-P130-1 and BGF 014-P137-2 cannot be considered as the best results for Z, as these results were found in non-scarified seeds and a thoroughly analysis and together with the parameters of total germination (%), this was extremely low (< 10,0%), making it clear that the few seeds that germinated, occurred at the same time, providing such a high index.

Among the evaluated genotypes, it is important to highlight the results found for the seeds of the Estilosantes Campo Grande cultivar, whose G and Z values did not show significant differences between the scarified and non-scarified seeds for all the analyzed descriptors. As the cultivar's seed production occurs on a large scale, harvesting is mechanized, followed by sieving and drying the seeds in the shade to reduce humidity (10-12%); then the seeds are submitted to pre-cleaning, ventilation and preparation for scarification in a processing machine. As a final product for commercialization, the result if a scarified seed (Fernandes *et al.*, 2000) and in this evaluation, the seeds showed a low performance after storage. It is worth mentioning that these seeds were purchased in 2011, in the local market and were stored under the same conditions as the accessions in the FGB-UEFS and because they are already scarified seeds, it is likely that the viability of the seeds was negatively affected.

It is worth mentioning that for the average germination time (Figure 1) there was no significant interaction, but in general it is important to consider that it took an average of approximately two days for the first seed to emit the root, demonstrating speed in the germination process. A characteristic of species that seek to establish themselves as quickly as possible or when environmental conditions are favorable, to the development of the new individual (Borghetti & Ferreira, 2004). Associated with the physiological conditions of the seeds of the accessions, it is worth mentioning that one of the centers of origin of *Stylosanthes* is the Brazilian Semiarid (Lewis *et al.*, 2005) and due to the climatic characteristics of this region, plants normally use this mechanism as a strategy for survival and development.

The speed and high percentage of germination of *Stylosanthes* spp. indicate longevity and vigor that were not lost in seeds in FGB-UEFS. Despite being stored for periods of seven to 13 years, they remained viable, demonstrating once again the efficiency of the storage procedure adopted.

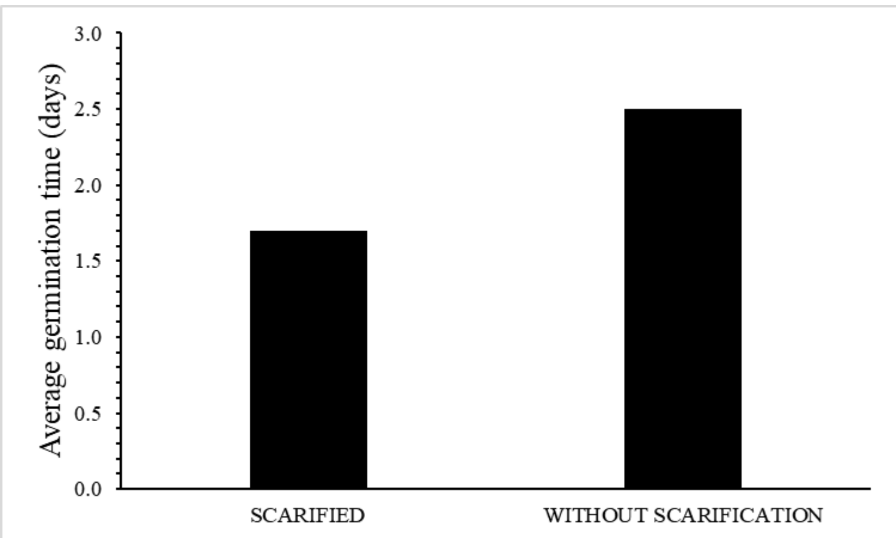

Figure 1: Average germination time of scarified and non-scarified *Stylosanthes* spp. seeds.

CONCLUSIONS

Seed storage following the methodology of Gómez-Campo (2006) is efficient for the conservation of *Stylosanthes* spp. with high viability of its seeds over the years for most accessions dependent on overcoming physical dormancy to reach expressive levels of germination, even after long periods of storage.

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