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Methods for overcoming dormancy in *Brachiaria brizantha* seeds

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Abstract

Brachiaria brizantha is a forage species widely used in Brazilian pastures and its seeds have contamination in several areas of countries. Its high germination is fundamental in pasture formation. However, it is common for seeds of this species to have natural dormancy, negatively influencing pasture formation, resulting in losses to the producer. Thus, the objective of this study was to determine a methodology to overcome dormancy of 'Marandu' and 'Piatã' *B. brizantha* seeds. Four batches from the 2016/2017 and 2017/2018 harvests were used. Initially, batch profile was evaluated by the following characteristics: determination of moisture content, weight of a thousand seeds, first germination count, germination, germination speed index, initial stand, emergency, emergency speed index and fungi incidence in seeds. The seeds were subjected to the following treatments to overcome dormancy: mechanical (removal of glume, palea and lemma), sulfuric acid (98%, 36 N), potassium nitrate (0.2%), heat treatment (70 °C and 85 °C, during 5h, 10h, 15h and 20h) and a control. The obtained results allow inferring that the causes of dormancy of 'Piatã' seeds are of a physical nature, with the tissues surrounding the seed being the main factor that prevent germination. The mechanical method and scarification with sulfuric acid the most efficient in overcoming dormancy. The mechanical treatment with removal of the glumella is efficient to overcome the dormancy of*B. brizantha* seeds, providing an increase in the germination percentage.

Keywords: forage, germination, quality.
Abbreviations: UFVJM_ Federal University of the Jequitinhonha and Mucuri Valleys; RSA_ Rules for Seed Analysis; GSI_germination speed index; ESI_emergence speed index; H_2SO_4_ Sulfuric acid; KNO_3_ Potassium nitrate.

Introduction

Brachiaria brizantha has shown promising results in agricultural systems, such as plant cover, being an important tool in the diversification of agricultural production systems (Machado et al., 2011). Thus, it is necessary that there is a rapid and uniform seedling emergence in the field. However, Lima et al. (2015) mention that seeds of the species *B. brizantha* present difficulties in germination, showing irregularity in maturation, shattering and dormancy, whose nature, intensity and persistence are not yet sufficiently known, directly interfering in the commercialization patterns of the seeds of this species since, according to Normative Instruction No. 30 (Brasil, 2010), the seed batches of *B. brizantha* must reach at least 60% germination.

Thus, dormant seeds need to undergo some pre-germination treatment for the transcription of the genetic message to occur and thus the activation of the metabolic sequence that culminates with germination. Since the initial events after imbibition in dormant seeds depend on the genetic information already existing during the maturation period, that is, prior to the beginning of the physiological rest (Marcos Filho, 2015).

Studies aimed at overcoming dormancy of *B. brizantha* seeds are indispensable for obtaining better quality seed batches, with high germination and vigor. In this context, pre-germination treatments can reduce the percentage of dormant seeds, accelerating germination and homogenizing seedling establishment (Batista et al., 2018; Pereira et al., 2018; Ribeiro et al., 2019).

Some methods are already recommended for overcoming seed dormancy (Abreu et al., 2017). However, the application and efficiency of these treatments depend on the intensity of dormancy, which varies widely among species, origins and years of collection (Alencar et al.,2009) and each test can influence, in different ways, the physiological quality of the seeds. For *B. brizantha* seeds, there are studies using chemical treatments with sulfuric acid (Silva et al., 2014; Lima et al., 2015) and potassium nitrate (0.2%) (Brasil, 2009; Batista et al., 2016), heat treatments (Martins and Silva, 2006; Lima et al., 2015) and mechanical treatment (Vale et al., 2017).

However, the choice of the method to be recommended to overcome seed dormancy, must take into account not only its efficiency, but also the risks that can occur to workers and the environment and, in addition, to avoid damage to seeds. In this context, there is still a need for studies that compare such methods, providing reliable information on the most appropriate procedure to be used to overcome dormancy of *B. Brizantha* seeds. Given the above, the objective of this study was to determine a methodology to overcome dormancy of 'Marandu' and 'Piatã'*B. brizantha* seeds.

Results and discussion

Variables of Brachiaria brizantha 'Marandu'

From the results, it was possible to observe that there were significant differences for the profile variables of cultivar Marandu, except for the initial stand and emergence speed index (Table 2).

The values of moisture content for 'Marandu' varied between 9.75% to 10.65% among the batches (Table 2). According to Novembre et al. (2006), the adequate value for the conservation of *Brachiaria* seeds ranges from 10% to 12%. For cultivar Marandu, there was a variation of 0.9% moisture among batches. A variation of up to 2% moisture among samples is tolerable and the value of each sample must be below 13% to avoid seed deterioration during storage (Marcos Filho, 2015), indicating that there was no interference of the water content in the results obtained in the other tests of this study. The moisture content is an important characteristic in the standardization of any method to assess the quality of seeds, as well as in the obtention of uniform results between laboratories and within the same laboratory (Oliveira et al., 2016).

For the weight of a thousand seeds (WTS), the*B. brizantha* seeds were considered small, since they have less than 200g, according to Brasil (2009). The weight of a thousand seeds in cultivar Marandu varied from 7.32g to 8.69g, acquiescing the moisture results, considering that batches 2 and 3 presented the highest averages for the two variables (Table 2), in agreement with Batista et al. (2016), in which they report that when the moisture content is higher, there is an increase in seed mass. The variation in the weight of a thousand seeds among batches indicates that there is a need for classification for commercialization, since the weight of a thousand seeds is used to calculate the sowing density and the weight of the working samples, in addition to information about the physiological maturity stage and seed conditions in relation to infestation by pathogens (Brasil, 2009).

Seed moisture and weight are of paramount importance in the germination process, since water availability is essential for the activation and maintenance of seed metabolism (Bewley et al., 2013) and the larger the seed, the greater the weight and the available reserves for germination (Carvalho and Nakagawa, 2012). The batches with the highest values of moisture and weight of a thousand seeds yielded the best results in the first germination count, germination speed index and germination. The difference of batches in terms of quality levels indicated the inferiority of batch 1, and placed batches 2 and 3 as superior, followed by batch 4 (Table 2). For cultivar Marandu, it can be observed that the batches reached a germination percentage between 11% and 57%, lower than the standard for commercialization which, according to Normative Instruction No. 30 (Brasil, 2010), is 60%.

In emergence, the batches provided a percentage ranging from 44% to 63%. The lowest percentage of emerged seedlings was found in batch 1. Batch 4 was the one that yielded the highest percentage of emerged seedlings, together with batches 2 and 3.

In all 'Marandu' batches, the values of the percentage of emergence were higher than germination, in accordance with Juliatti et al. (2011), who observed that the emergence test using the mixture of soil and sand as a substrate provides superior results in relation to the germination percentages obtained in the laboratory test. According tothe authors, this fact probably occurs due to the escape mechanism, in which the seedling, when emerging, releases the infected integument into the soil whereas, in the germination test using paper substrate, the integument infected by pathogens remains associated with the seeds, causing deterioration.

When this happens in pasture formation areas, in addition to being a means of disseminating the pathogens, the seed can cause the formation of infected seedlings and, consequently, generate losses to the producer. This fact emphasizes the importance of identifying pathogens associated with seeds, in order to provide subsidies for the establishment of field health standards, tolerance of pathogen seed infections and the adoption of efficient techniques for seed treatment.

As shown in Table 3, the pathogens that had the highest incidence in 'Marandu' batches were *Drechslera* sp., *Fusarium* sp. and *Phoma* sp., being considered of high incidence, once they are present in more than 3% of the seeds, with greater incidence in batches 1 and 4. The incidence of *Drechslera* sp. ranged from 3.00% to 12.25% among batches, like the incidence found for *Fusarium*sp., 4.12% to 12.75%. The highest incidence was observed for *Phoma* sp., ranging from 8.25% to 18%. Such fungi have already been reported in other studies with *Brachiaria* brizantha due to their high incidence (Silva et al., 2019).

The high frequency and incidence of these fungi are worrisome, as most of them have been described as potentially pathogenic to tropical forage grass plants (Mallmann et al., 2013; Witt et al., 2015). Fungi like *Drechslera* sp., *Fusarium* sp. And *Phoma*sp. could reduce seed viability, affecting seedling emergence and death (Vechiato et al., 2010). When associating the values of incidence of these fungi to the results of the physiological analyses of the profile of the batches (Table 2), it can be observed that the batches with the highest incidence of these fungi are those that demonstrate a lower quality.

According to Pazos et al. (2011), the fungus *Drechslera* sp. was classified as the main pathogen in the genus *Brachiaria* in a seed analysis laboratory in San Lorenzo in Paraguay. The occurrence of *Fusarium* sp. in seed batches is also worrying, since this fungus is capable of causing the death of seeds and plants of several species of economic importance (Salgado-Neto et al., 2016), its presence in a seed batch makes it impossible, even when present at low levels, due to its high infection potential (Ramos et al., 2014).

As for the infection of *Brachiaria* seeds by *Phoma* sp., it becomes a serious barrier to seed export to countries such as Mexico and Colombia (Martins et al., 2017). According to Avelino et al. (2019), infection by *Phoma* sp. can cause leaf spots with elongation, necrosis and irregular characteristics, rot, in addition to causing seed impracticability and death of seedlings. Once it has fast and aggressive growth, it can even kill infected seeds before germination (Mallmann et al., 2013).

In addition to the fungi already mentioned, others also occurred in smaller quantities, such as *Pithomyces*sp *Curvularia* sp, *Alternaria*sp *Aspergilus* sp., *Mucor* sp., *Cladosporium* sp. And *Rhizopus* sp. (Table 3). Infection with *Pithomyces* sp. And *Alternaria* sp. were considered to have a high incidence only in batch 1. For *Aspergilu s*sp. And *Mucor* sp., greater intensities were also observed in batch 1 (2.00% to 0.85%, respectively), being considered of medium incidence. Infection with *Cladosporium* sp. was considered to have a high incidence for batch 2 (3.50%) and a medium incidence for the other batches. For *Rhizopus* sp., there was a high incidence for batches 1, 3 and 4, ranging from 3.62% to 7.75%, and with a medium incidence in batch 2 (1.25%).

Batch 1 had the highest incidence for most fungi and was the one that showed the lowest physiological quality in the profile analyses. The sowing of these seeds associated with these fungi will cause losses to the producer, as it contributes to their dissemination to other areas, and will cause significant losses in fields.

In addition to the difference in the physiological and sanitary quality of the batches, the low germination percentage can be attributed to the presence of dormancy in *B. brizantha* seeds since, in this species, dormancy is the main factor of low germination. (Cardoso et al., 2014). This information is reinforced by the results obtained in this study since, regardless of batch quality, all had a low germination percentage. There is a need of a treatment for overcoming dormancy for the use of *B. brizantha* seeds, aiming at increasing the germination percentage and, consequently, resulting in the formation of more uniform pastures.

From Table 4, it is possible to observe the results of the germination test after the thermal treatment to overcome the dormancy of cultivar Marandu. It is possible to show that there were significant differences between the combinations of temperature and exposure time in the germination percentage of the batches.

There were different levels of influence of the heat treatment in overcoming the dormancy of ‘Marandu’ seeds. However, the combinations of 70ºC for 5 and 15 hours, were among the best heat treatments to reduce seed dormancy in all batches, with greater influence on batches 2 and 3 (Table 4).

The best responses with the use of 70ºC for 5 and 15 hours, were also observed by other authors in overcoming dormancy in *B. brizantha* seeds: 70 °C for 5, 10 and 15 hours (Martins and Silva, 2001), 70 °C for 15 hours (Martins e Silva, 2003), 70 °C for 5 hours (Martins e Silva, 2006). However, the germination percentage with the use of heat treatment using dry heat, was below the standard for commercialization, indicating that the use of such a method depends on the seed dormancy level, since this method has already been efficient in overcoming dormancy in other studies with *B. brizantha* (Martins and Silva, 2006).

When comparing the different methods to overcome seed dormancy in ‘Marandu’ batches, it was possible to verify that there was a significant difference between the methods for all batches (Table 5).

The germination percentage of cultivar Marandu using the heat treatment (70ºC/5hours) was similar to the control, except for batch 1 (Table 5). The use of KNO_3treatment was lower than the other treatments. The action of KNO_3in overcoming dormancy is associated with its performance as an oxidizer and electron acceptor. KNO_3stimulates the pentose phosphate pathway and the shikimic acid pathway by initiating metabolic reactions that culminate in cytochrome acidosis in the Krebs cycle, in addition to being vital for the biosynthesis of essential amino acids (Cardoso et al., 2015) and, consequently, in the supply of energy and raw material for the development of the embryonic axis (Menezes and Mattioni, 2011). However, Bonome et al. (2006) reported that this low molecular weight salt can penetrate the tissues of brachiaria seeds, leading to phytotoxicity, causing a harmful effect on germination.

The highest germination percentage of cultivar Marandu (Table 5), in all batches, was obtained with the use of treatments with removal of glumes and the immersion of seeds in sulfuric acid (H_2SO_4). With the exception of batch 1, the batches that were subjected to treatments, mechanical and immersion of seeds in H_2SO_4, yielded a percentage above the minimum standard for commercialization (60%) required for the species (Brasil, 2010).

When companies overcome the dormancy of *B. brizantha* seeds, they frequently scarify the seed tegument, which is a more laborious method and can also cause damage to the seeds depending on the depth. The results of this study show that there is no need to scarify the tegument, since only the removal of glume, palea and lemma is sufficient to overcome the dormancy of ‘Marandu’ *B. brizantha*.

The results of the mechanical treatment and immersion in H_2SO_4indicate that the presence of dormancy in ‘Marandu’ *B. brizantha* seeds has as its fundamental cause the tissues that surround the seed, such as glume, lemma and palea, once both the mechanical treatment and scarification with H_2SO_4have the objective to improve seed permeability to water, gas exchange and, eventually, radicle protrusion, as some have wraps that prevent or delay germination.

When observing the development of seedlings submitted to the different treatments to overcome dormancy of cultivar Marandu (Figure 2), it is possible to verify that there is a difference in the aspect of the seedlings that were submitted to the sulfuric acid treatment in relation to the other treatments. Despite being able to reduce seed dormancy, the use of H_2SO_4 provided the formation of apparently less vigorous seedlings. This fact can be confirmed based on the reports by Cardoso et al. (2014) and Lima et al. (2015), who describe that H_2SO_4can compromise the internal structures of the seed and accelerate the deterioration process; consequently, it can yield less vigorous seedlings, in addition to presenting risks for workers and the environment.

Characteristics of Brachiaria brizantha ‘Piatã’

The results for the profile characteristics of ‘Piatã’ batches are shown in Table 6, with a significant difference in all variables.

In ‘Piatã’, the moisture percentage values of the batches varied from 8.65% to 10.72% (Table 6), showing a variation of 2% among batches, staying within the tolerable variation among samples, which is 2% (Marcos Filho, 2015). For the weight of a thousand seeds, there was no relationship with

Table 1. Commercial batches of *B. brizantha* seeds from different production regions in Brazil.

'Marandu'*B. brizantha*		
Batch	Production site	Harvest
L1	Minas Gerais	2016/2017
L2	São Paulo	2016/2017
L3	Mato Grosso	2016/2017
L4	São Paulo	2016/2017
'Piatã' *B. brizantha*		
L1	Minas Gerais	2016/2017
L2	São Paulo	2016/2017
L3	Mato Grosso	2016/2017
L4	São Paulo	2017/2018

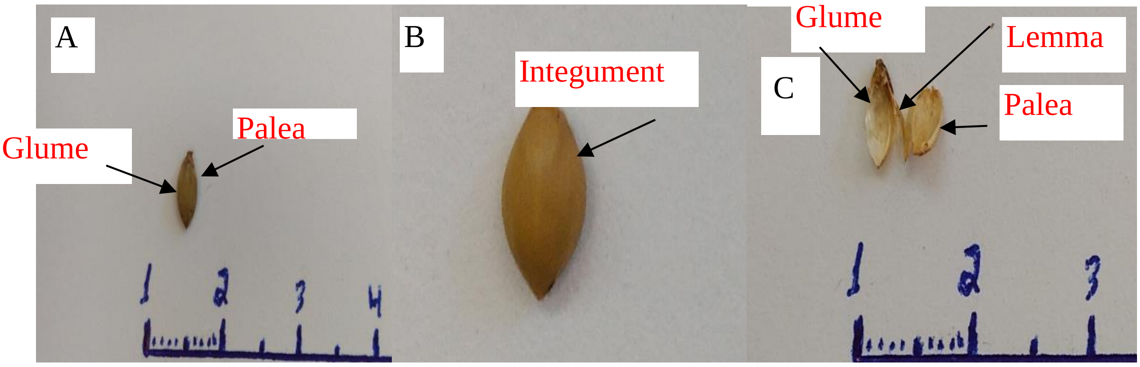

Fig 1. Pictures of 'Marandu' *B. brizantha*; A) whole seed, B) bare seed (without glume, lemma and palea), C) glume, lemma and palea.

Table 2. Percentages of moisture content (C), weight of a thousand seeds (WTS), first germination count (FC), germination (G), germination speed index (GSI), initial stand (IS), emergence (E) and emergence speed index (ESI) of the four seed batches of 'Marandu' *B. brizantha*.

Batches	Tests							
	C (%)	WTS (g)	FC (%)	G (%)	GSI	IS (%)	E (%)	ESI
L1	9.75 b	7.32 c	9 c	11 c	1.24 d	33 a	44 b	4.07 a
L2	10.40 a	8.14 b	49 a	57 a	6.07 b	37 a	59 a	5.02 a
L3	10.65 a	8.69 a	48 a	50 a	8.40 a	54 a	59 a	6.80 a
L4	10.10 b	7.41 c	34 b	39 b	3.82 c	42 a	63 a	5.11 a
CV %	3.77	4.10	16.66	15.52	15.38	15.94	13.87	9.18

*Means followed by the same letter in the column do not differ, and belong to the same group by the Scott-Knott test at 5% probability.

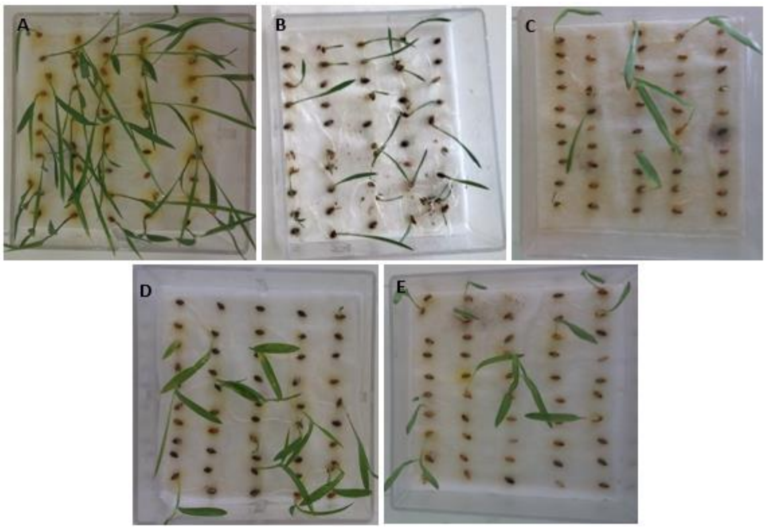

Figure 2: Photos of the germination test for 'Marandu' *B. brizantha*submitted to different methods to overcome dormancy: A) mechanical, B) sulfuric acid, C) potassium nitrate, D) heat treatment and E) control.

Table 3. Percentage of fungi incidence in the four seed batches of ‘Marandu’*B. brizantha*.

Batches	Incidence of Fungi (%)				
	*Drechslera*sp.	*Fusarium*sp.	*Phoma*sp.	*Pithomyces*sp.	*Curvularia*sp.
1	12.25 a	12.75 a	18.00 a	3.12 a	1.75 a
2	4.25 b	4.25 b	8.50 b	0.25 c	0.25 c
3	3.00 b	4.12 b	8.25 b	0.00 c	2.12 a
4	10.62 a	10.12 a	14.25 a	0.87 b	1.00 c
CV (%)	13.76	16.70	17.50	24.35	23.83
Batches	*Alternaria*sp.	*Aspergilus*sp.	*Mucor*sp.	*Cladosporium*sp.	*Rhizopus*sp.
1	3.10 a	2.00 a	0.85 a	1.25 b	3.87 b
2	0.00 c	0.00 c	0.00 b	3.50 a	1.25 c
3	0.50 b	0.00 c	0.00 b	1.87 b	3.62 b
4	0.00 c	0.50 b	0.00 b	2.37 a	7.75 a
CV (%)	18.81	20.45	14.50	19.89	17.44

*Means followed by the same letter in the column do not differ and belong to the same group by the Scott-Knott test at 5% probability.

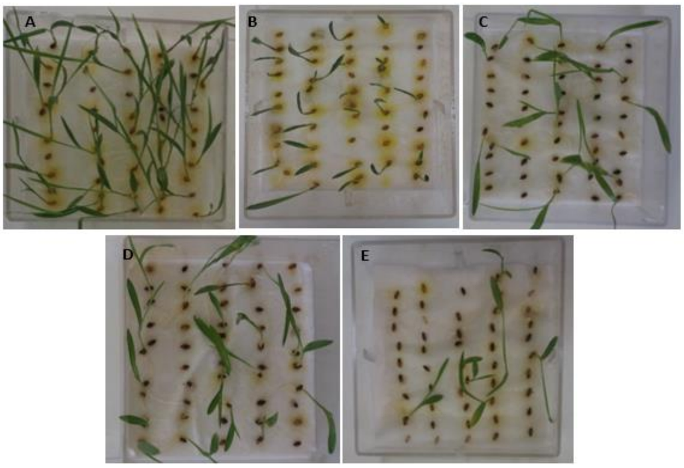

Fig 3. Photos of the germination test for ‘Piatã’ *B. brizantha*submitted to different methods to overcome dormancy: A) mechanical, B) sulfuric acid, C) potassium nitrate, D) heat treatment and E) control.

Table 4. Germination percentage (%G) of the four ‘Marandu’ *B. brizantha* batches subjected to heat treatments to overcome dormancy.

Heat treatments	Batches			
	L1	L2	L3	L4
	Germination (%)			
70 ºC/5hours	26 aB	57 aA	48 aA	37 aB
70 ºC/10hours	17 aB	42 bA	35 bA	27 bB
70 ºC/15hours	22 aB	51 aA	49 aA	41 aA
70 ºC/20hours	0 cB	5 cA	7 cA	3 dA
85 ºC/5hours	10 bB	16 cB	32 bA	24 bA
85 ºC/10hours	7 bB	33 bA	28 bA	16 cB
85 ºC/15hours	4 bB	32 bA	43 aA	16 cB
85 ºC/20hours	5 bC	35 bA	30 bA	16 cB
CV (%)	18.49			

*Means followed by the same letter in the column do not differ, and belong to the same group by the Scott-Knott test at 5% probability.

Table 5. Germination percentage (G) of the four ‘Marandu’*B. brizantha*batches subjected to different treatments to overcome dormancy.

Treatments	Batches			
	L1	L2	L3	L4
	Germination (%)			
Mechanical	58 aB	69 aA	67 aA	74 aA
Sulfuric acid	54 aB	71 aA	62 aB	79 aA
Potassium nitrate	7 cC	35 cA	40 cA	20 cB
70 ºC/5hours	21 bC	59 bA	50 bA	39 bB
Control	11 cC	57 bA	49 bA	39 bB
CV (%)	13,60			

*Means followed by the same letter in the column do not differ, and belong to the same group by the Scott-Knott test at 5% probability.

Table 6. Percentages of moisture content (C), weight of a thousand seeds (WTS), first germination count (FC), germination (G), germination speed index (GSI), initial stand (IS), emergence (E) and emergence speed index (ESI) of the four seed batches of 'Piatã' *B. brizantha*.

Batches	Tests							
	C (%)	WTS (g)	FC (%)	G (%)	GSI	IS (%)	E (%)	ESI
L1	10.45 b	10.8 a	34 a	35 a	3.30 b	58 a	72 a	7.73 a
L2	10.15 c	9.06 d	7 b	9 b	0.51 c	26 b	46 b	3.61 b
L3	10.72 a	9.85 c	41 a	40 a	7.55 a	62 a	68 a	8.11 a
L4	8.65 d	10.45 b	39 a	47 a	4.72 b	46 a	77 a	6.36 a
CV %	1.15	2.12	17.33	10.42	17.80	14.66	15.51	9.18

*Means followed by the same letter in the column do not differ, and belong to the same group by the Scott-Knott test at 5% probability.

Table 7. Percentage of fungi incidence in the four seed batches of 'Piatã'*B. brizantha*.

Batches	Incidence of Fungi (%)			
	Drechslera sp.	*Fusarium* sp.	*Phoma* sp.	*Pithomyces* sp.
1	1.25 c	4.25 b	3.00 c	0.50 c
2	9.75 a	11.62 a	12.75 a	5.75 a
3	0.00 c	4.75 b	5.50 b	2.25 b
4	4.75 b	6.50 ab	5.25 b	1.25 b
CV (%)	19.04	17.87	19.50	17.81
Batches	*Curvularia* sp.	*Aspergilus* sp.	*Cladosporium* sp.	*Rhizopus* sp.
1	0.50 c	0.90 b	1.00 b	1.25 a
2	0.37 c	21.50 a	25.75 a	0.00 b
3	2.37 a	1.50 b	1.53 b	0.00 b
4	1.25 b	2.12 b	2.62 b	0.00 b
CV (%)	25.00	17.82	18.87	7.26

*Means followed by the same letter in the column do not differ, and belong to the same group by the Scott-Knott test at 5% probability.

Table 8. Germination percentage (G) of the four 'Piatã'*B. brizantha* batches subjected to heat treatments to overcome dormancy.

Heat treatments	Batches			
	L1	L2	L3	L4
	Germination (%)			
70 ºC/5hours	41 bA	8 aC	22 bB	41 bA
70 ºC/10hours	55 aA	8 aC	35 aB	43 bB
70 ºC/15hours	40 bB	6 aC	29 aB	55 aA
70 ºC/20hours	43 bA	2 bC	14 bB	39 bA
85 ºC/5hours	56 aA	11 aC	36 aB	51 aA
85 ºC/10hours	58 aA	12 aC	37 aB	60 aA
85 ºC/15hours	46 bA	5 bC	17 bB	23 cB
85 ºC/20hours	49 aA	7 aC	21 bB	55 aA
CV (%)	10.81			

*Means followed by the same letter in the column do not differ and belong to the same group by the Scott-Knott test at 5% probability.

Table 9. Germination percentage(G)of the four 'Piatã'*B. brizantha* batches subjected to different treatments to overcome dormancy.

Treatments	Batches			
	L1	L2	L3	L4
	Germination (%)			
Mechanical	78 aA	46 aB	76 aA	77 aA
Sulfuric acid	55 aA	49 aA	59 aA	68 aA
Potassium nitrate	41 bA	5 bB	39 bA	23 bA
85 ºC/10hours	56 aA	13 bC	36 bB	67 aA
Control	32 bA	11 bB	31 bA	40 bA
CV (%)	18.86			

*Means followed by the same letter in the column do not differ and belong to the same group by the Scott-Knott test at 5% probability.

the result of moisture content between batches, with the highest WTS value observed in batch 1, followed by batches 4, 3 and 2, respectively (Table 6).

For the physiological quality studies, it was observed, by the percentage of normal seedlings obtained in the tests of first germination count, germination speed index, germination, initial stand, emergence speed index and emergence, that there were significant differences between 'Piatã' seed batches (Table 6). The results indicate that batches 1, 3 and 4 are of superior quality, and batch 2 of inferior quality. In the germination test of cultivar Piatã, the batches yielded a percentage of normal seedlings ranging from 9% to 47%, not reaching the minimum percentage for commercialization of 60% (Brasil, 2010) indicating that, in addition to the quality difference among batches, the presence of dormancy is one of the factors that prevent the germination of these seeds.

The percentage of the germination test of 'Piatã' batches was lower than the percentage of the emergence test (Table 6). This result may be related to the association of seeds with pathogens, since the conditions of the germination test are more favorable for infection (Juliatti et al., 2011).

Through the health analysis of 'Piatã' batches, the incidence of fungi *Drechslera* sp., *Fusarium* sp., *Phoma* sp., *Pithomyces* sp., *Curvularia* sp., *Aspergilus* sp., *Cladosporium* sp. And *Rhizopus* sp. was observed (Table 7). Among the fungi identified in cultivar Piatã, all were found in the seeds of cultivar Marandu, in addition to beingre ported in other studies with *B. brizantha* seeds (Pazos et al., 2011; Sbalcheiro et al., 2014), indicating that such fungi are commonly found associated with the seeds of this species.

Infection by *Drechslera* sp. was considered to have a high incidence only in batches 2 and 4. For *Fusarium* sp. And *Phoma* sp., a high incidence was found in all batches, as they had an incidence above 3%. The incidence of *Pithomyces* sp. was considered high only in batch 2 (5.75%), with a medium incidence in batches 3 and 4, and low in batch 1. The lowest incidence was observed for *Curvularia* sp. E *Rhizopus* sp.; therefore, these fungi had little or no interference in batch germination.

Regarding the association of seeds with fungi *Aspergilus*sp. And *Cladosporium* sp., there was similarity between the results, with a high incidence in batch 2, (21.50% and 25.75%, respectively). In the other batches, medium incidences of these fungi were verified, ranging from 0.90% to 2.62% (Table 7).

When analyzing fungi incidence and batch germination (Tables 6 and 7), it is possible to observe that batch 2, which yielded the lowest germination percentage, was also the one with the highest incidence levels for most fungi. Thus, it is evident that, in this study, the pathogens present influenced the seed germination capacity, proving that health is an attribute that is directly linked to seed quality. According to Mallmann et al. (2013), the use of seeds of low health quality has been a frequent cause of failure in the formation of areas with pastures, negatively impacting the sustainability of livestock activity. Thus, the results obtained in this study are important to provide subsidies for the implementation of a seed certification program for health in *Brachiaria brizantha*, the main forage grass in Brazil.

Regarding dormancy overcoming of cultivar Piatã, for the heat treatment (Table 8), the use of 85ºC for 5 and 10 hours were the combinations that provided the best results in overcoming the dormancy of the batches, resulting in higher percentages of final germination. In practical terms, the shorter time of seed exposure is a favorable factor to indicate the best methodology; however, the use of 85ºC for 10 hours provided higher germination rate values and, in batch 4, the minimum rate recommended for commercialization (60%) was reached (Brasil, 2010). Therefore, this combination can be more efficient to overcome the dormancy of 'Piatã' seeds. In the literature, it has been observed that, for the heat treatments in *B. brizantha*, the best results have been obtained with the use of temperatures between 70ºC to 85ºC, in periods between 5 to 15 hours (Almeida and Silva, 2004).

The influence of different treatments in overcoming seed dormancy in 'Piatã' batches is shown in Table 9. In batches 1 and 4, the treatment with removal of glumes, sulfuric acid and thermal (85 ºC/10 hours), did not differ from each other. The treatment with removal of glumes and sulfuric acid were significantly similar in all batches.

The final germination percentage of 'Piatã' seeds shows the efficiency of the mechanical treatment with removal of glumella in overcoming dormancy of *B. brizantha*. With the exception of batch 2, glume removal provided a germination percentage above that recommended for commercialization (60%) (Brasil, 2010). For the other treatments, only sulfuric acid and the heat treatment (85ºC/10 hours), in batch 4, provided a germination rate above that recommended for commercialization. The rapid seed germination is important in pasture formation, as it allows rapid development early in the growth stages, reducing the appearance of weed plants and maximizing the interception of light.

In addition to obtaining a higher germination rate with the mechanical treatment only with the removal of glumella, seedlings with more vigorous aspects are formed in relation to the chemical treatment with sulfuric acid (Figure 3), in agreement with Cardoso et al. (2014) and Lima *et al.* (2015), who report that sulfuric acid can cause damage to seeds, and thus yield less vigorous seedlings. This fact emphasizes the importance of choosing the correct treatment to overcome dormancy, since more vigorous seedlings have greater potential to develop in field conditions.

The obtained results allow to infer that the causes of dormancy of 'Piatã' seeds are of a physical nature, with the tissues surrounding the seed being the main factor that prevent germination, making the mechanical method and scarification with sulfuric acid the most efficient in overcoming dormancy. Such results corroborate the hypothesis that the main type of dormancy in *B. brizantha* is attributed to the envelopes (glume, palea and lemma), which constitute a barrier to germination due to the restriction to the movement of water, restriction to gas exchange and mechanical restriction (Cardoso et al., 2014; Silva et al., 2014).

Materials and Methods

Location and study material

The study was conducted at the Seed Laboratory of the Department of Agronomy of Universidade Federal dos Vales do Jequitinhonha e Mucuri – UFVJM, Diamantina, MG.

Four commercial batches of 'Marandu' and 'Piatã' *B. brizantha*seeds, from different production regions, were used, as shown in Table 1.

In the laboratory, the batches were stored in a cold chamber (10 ºC and 50% RH) to avoid loss of quality during the experimental period. The seeds were subjected to processing to remove impurities, using an air column blower (South Dakota) to separate the pure seed fraction from the batches. Subsequently, they were homogenized to obtain the average working sample, according to the Rules for Seed Analysis (RSA) (Brasil, 2009).

Evaluated variables

For batch characterization, the following determinations and tests were carried out:

Seed moisture content

It was obtained using the greenhouse method, at 105ºC, for 24 hours (Brasil, 2009). Four replications were used, with a sample weight of 5 g.

For the weight of a thousand seeds, the methodology described by Brasil (2009) was used, in which eight replications of 100 seeds were weighed in an analytical balance and the standard deviation and the variation coefficient were calculated; the results were expressed in grams.

The germination test was performed with four replications of 50 seeds per batch, sown on three sheets of germitest paper moistened with distilled water, in the amount of 2.5 times the weight of the substrate, in gerbox, and stored in a BOD chamber (Brasil, 2009) at 30 ºC (Vieira et al., 1998). The evaluations were carried out on the 7th day (first germination count) and finished on the 21st day (final count), computing normal seedlings. The germination speed index (GSI) was obtained by daily computing the germinated seeds and calculated according to Maguire (1962).

In the seedling emergence test, 50 seeds from each batch were used with four replications. The seeds were sown in plastic boxes containing 2:1 soil and sand, moistened with distilled water. The boxes were kept in a growth room at 25 ºC with constant light. After the beginning of emergence, assessments were performed daily, computing the initial stand on the 7th day. The test finished after the emergence percentage stabilized, evaluating the number of normal seedlings that emerged. The emergence speed index (ESI) was determined according to the Maguire formula (1962).

The health test was performed using the filter paper method (blotter test), using 2,4-D (5ppm) and freezing. 400 seeds were used, divided into 8 replications of 50 seeds arranged in gerbox, on three sheets of filter paper soaked with distilled water, 2,4-D and agar. The gerboxes were then capped and kept at -8 ºC for 24 hours and incubated in a B.O.D. at 25 ºC, with a photoperiod of 12 hours and for 17 days. After this period, the seeds were examined individually, with the aid of a magnifying glass and a stereoscopic microscope, in order to identify the presence of fungi. The result was expressed as percentage of infected seeds. To interpret the results, the detected fungi were divided into three categories based on the incidence: (i) high, higher than 3%; (ii) average, between 0.5 and 2.8%; and (iii) low, below 0.5% (Melo et al., 2017).

To evaluate if seed dormancy was overcome, the following methodologies were tested:

Sulfuric acid (H_2SO_4): The seeds were immersed in sulfuric acid (98%, 36N) concentrated for 15 minutes and then washed in running water for 5 minutes and dried in the shade.

Potassium nitrate (KNO_3)

The seeds were sown on a substrate moistened with KNO_3(0.2%).

Mechanical scarification

Seed glume, lemma and palea were manually removed. The seeds were then disinfected in 0.5% sodium hypochlorite solution for five minutes and washed in distilled water.

Heat treatment

First, a pre-test was carried out with seed exposure to 70 °C and 85 °C, for 5, 10, 15 and 20 hours in an air circulation oven. Subsequently, it was selected by comparing the means, using the Scott-Knott test at 5% probability, the best combination of temperature and period of seed exposure. The best thermal method was then compared with the other methods for overcoming dormancy, following the factorial scheme 4 x 4 (four seed batches for each cultivar x four methods for overcoming dormancy).

Statistical design and analysis

For the experiment, a completely randomized design was adopted. The results of the analyses of the profile of the batches, health and overcoming dormancy, were submitted to the analysis of variance and, when significant, the means were grouped together by the Scott-Knott test, at 5% probability. Statistical analyses were performed with the aid of the statistical software "R" (R CORE TEAM, 2013).

Conclusions

For 'Marandu' and 'Piatã'*Brachiaria brizantha*, the mechanical method with removal of the glumella is the most recommended to overcome seed dormancy.

Conflicts of interest

The authors declare no conflicts of interest.

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