

SEED CONDITIONING PROCESS IN THE SANITARY QUALITY OF MARANDU GRASS SEEDS

BENEFICIAMENTO NA QUALIDADE SANITÁRIA DE SEMENTES DE CAPIM-MARANDÚ

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ABSTRACT: The purpose of seed conditioning process is to separate seeds and their associated structures into different fractions and retain only good and healthy seeds. Thus, the aim of this study was to evaluate the effects the stages of seed conditioning have on the sanitary quality of marandu grass seeds. The seeds were sampled before and during the seed conditioning process, i.e., after exiting the air-screen cleaner and sieves (seeds discharged from the top, middle and bottom sieves), the first gravity separator (seeds drifting around the table, upper and intermediate discharge) and second gravity separator (upper, intermediate and lower discharge). The sanitary analysis was performed using the filter paper method with and without surface disinfestation of the seeds, which were incubated at $20\pm 2^{\circ}\text{C}$, with a photoperiod of 12 hours for seven days. The experiment was conducted in a completely randomized design, in a 2x6 factorial scheme (surface disinfestation x stages of the seed conditioning process) and ten replications. The data was submitted to analysis of variance, using the F test and, when significant, the means of the treatments were compared using the Tukey test at 5% probability. It was concluded that it is not possible to improve the sanitary quality of marandú grass seeds through the seed conditioning process.

KEYWORDS: Blotter test. *Brachiaria brizantha*. Seed sanity. Seed pathology.

INTRODUCTION

Seed conditioning allows for the improvement of the sanitary quality of the seeds by eliminating those infested with phytopathogens (MARCHI et al., 2010). However, this process can also increase the chances of contamination as the soil in the seed lots may be contaminated with reproductive fungi. Thus, when put inside the machines to be conditioned, the seeds could already be contaminated, as was verified in *Panicum maximum* cv. Tanzania and cv. Mombaça (MELO et al., 2017; SILVA et al., 2019).

Discharges from the conditioning of the fungi, as these materials are usually acquired from low-tech forage seed companies to be mixed with the seed lots and to serve less demanding markets (HESSEL et al., 2012). Moreover, the seed conditioning plants usually contain a high amount of suspended dust in the air, which could result in the machines and seeds being contaminated by spores present in the dust particles (MELO et al., 2017).

Thus, it can be inferred that for better sanitary quality of seeds, there must be an improvement in the conditioning process of forage

grass seeds (MARCHI et al., 2010). Tropical forage seeds may be an important vehicle for the spread of pathogens among different regions and few is known about their sanitary quality (VECHIATO; APARECIDO; FERNANDES, 2010). The presence of pathogens in the seeds results in a decrease in germination and vigor, which in turn, compromises the development and formation of the crop in the field (MARCHI et al., 2010; MALLMANN et al., 2013; MARCOS et al., 2015; MARTINS et al., 2017).

The main factors that have contributed to the production and commercialization of contaminated forage seed lots is the demand, by product quality (MARCHI et al., 2010).

However, with the expansion of pastures and intensification of livestock activity in recent years several diseases have begun to occur in pastures, increasing concerns about seed sanity. In addition, pathogens associated with these diseases have become significantly important in producing regions (MARCHI et al., 2009; MALLMANN et al., 2013).

The incidence of some potentially pathogenic fungi was reported in seeds of *Panicum*

maximum and *Brachiaria* spp. such as *Curvularia* sp., *Phoma* sp., *Fusarium* sp., *Exserohilum* sp. (syn. *Helminthosporium* sp.), *Cercospora* sp., *Colletotrichum* spp., *Drechslera* sp., *Alternaria* sp., *Rhizoctonia* spp. (VECHIATO; APARECIDO; FERNANDES, 2010; SILVA et al., 2019). Fungi like *Curvularia* sp., *Phoma* sp., and *Exserohilum* sp. are responsible for diseases that cause rot, non-viable seeds and seedling death (MARCHI; FERNANDES; VERZIGNASSI, 2011).

Secondary metabolites and storage fungi, such as *Aspergillus* sp., *Cladosporium* sp., *Epicoccum* sp., *Nigrospora* sp., *Penicillium* sp. and *Trichoderma* sp., can be found in a lower incidence in *P. maximum* and *Brachiaria* spp. seeds. (MARCHI et al., 2010; MALLMANN et al., 2013; MARCOS et al., 2015; WITT et al., 2015).

The presence of pathogens may decrease the germination percentage, thus, preventing the seed lots from being sold or traded. In addition, these pathogens could be a barrier to exportation, due to the phytosanitary laws of certain countries. As seeds are a vehicle for dissemination, most countries that import forage seeds impose restrictions to Brazil (TSUHAKO, 2009). Thus, the aim of this study was

to evaluate the effect of each stage of seed conditioning process has on the sanitary quality of marandu grass seeds.

MATERIAL AND METHODS

Marandu grass seeds were collected by an agricultural sweeper in certified seed production fields located in Monte Alegre de Minas, MG, Brazil. According to Alvares et al. (2014) the climate of the region is classified as Aw, with an average annual temperature of 22.4°C and rainfall of 1313mm (INMET, 2017).

The seeds were processed in a seed conditioning plant for forage grasses by passing through an air-screen and sieves and two gravity separators measuring 2.40m long and 1.25m wide. The vibration velocity of the gravity separators was 1,750rpm, and the transverse and longitudinal inclination were 17° and 12°, respectively.

Samples were taken from the materials, as well as from the different machines, at each stage of the seed conditioning process resulting in 10 treatments (Figure 1).

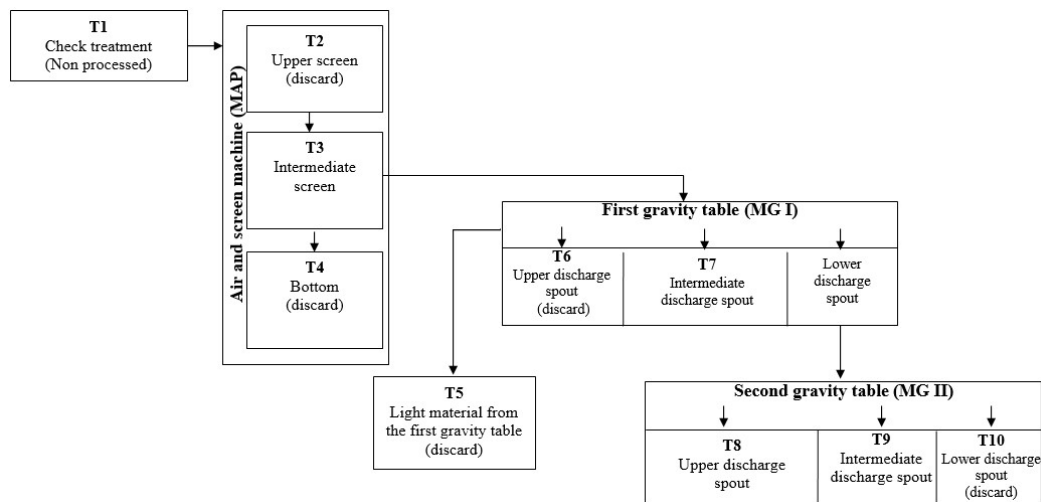


Figure 1. Flow diagram of seed conditioning process with points showing where the samples of marandu grass seeds were taken from, comprising the ten treatments.

Which treatments are described as: T1 - the control composed of the unprocessed seeds; T2 - intermediate sieve from the air-screen and sieves, eight mesh and 18mm bore; T3 - lower sieve of the air-screen and sieves, of 12 mesh of 26mm bore; T4 - bottom of the air-screen and sieves, which were not retained in the sieves; T5 - from the first gravity separator, removed by vacuum machine at the entrance; T6 - upper discharge of the first gravity separator, collected at 40cm from the highest end of the separator outlet, when considering the lateral

slope of the table; T7 - intermediate discharge of the first gravity separator, in the intermediate segment of 50cm from separator outlet; T8 - upper discharge of the second gravity separator, in the segment of 55cm from the upper end of the separator outlet; T9 - intermediate discharge of the second gravity separator, in the intermediate segment of 40cm; T10 - lower discharge of the second gravity separator, in the segment 30cm from the lower end (Figures 1, 2 and 3).

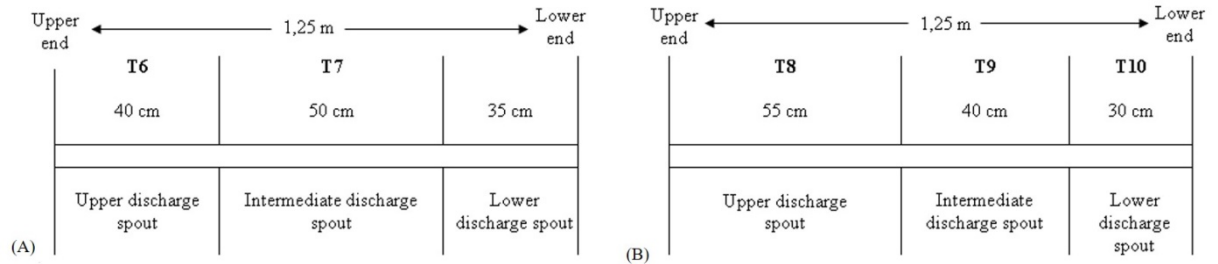


Figure 2. Sample collection points of *Brachiaria brizantha* cv. Marandu from the first (A) and second gravity separator (B).

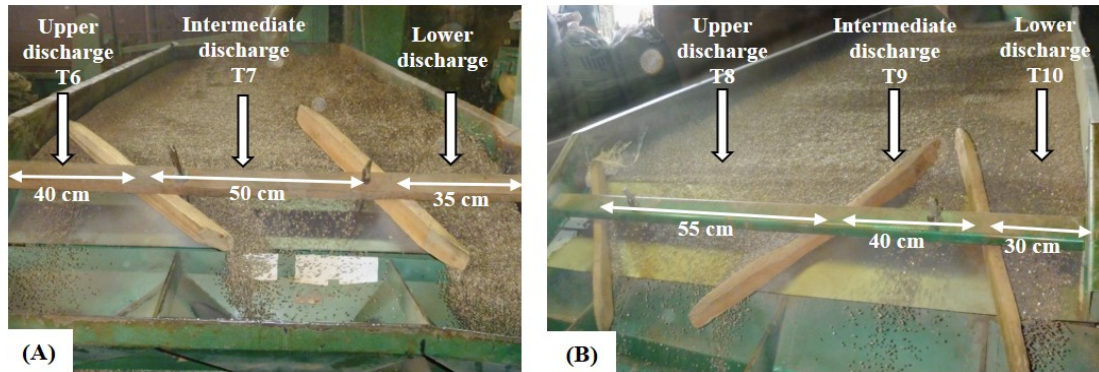


Figure 3. Sample collection points from the first (A) and the second gravity separator (B).

The divisions of discharge system of the two gravity separators were adjusted in order to allow for a higher concentration of undesirable materials to pass through the lower discharge, which is why they presented a variety of different sizes.

After stabilizing the machines, the seeds of each treatment (conditioning stage) were sampled at regular intervals, five minutes between each repetition, with 20 simple samples at an average weight of 100g for each treatment being obtained from different discharge nozzles of the machines (MELO et al., 2016a,b; 2017). Samples obtained in each treatment were grouped and homogenized in composite samples and reduced to form average samples of 500g each (BRASIL, 2009).

The samples were then taken to the seed analysis laboratory of Department of Plant Production - Plant Science, Faculty of Agrarian and Veterinary Sciences, Universidade Estadual Paulista "Júlio de Mesquita Filho", in Jaboticabal, SP, Brazil, which were reduced in a seed divider to obtain the study sample.

Sanitary analysis was carried out in the Laboratory of Seed Pathology of the Department of Phytosanitary of the Faculty of Agrarian and Veterinary Sciences - (UNESP), in Jaboticabal Campus, SP, Brazil. Ten replications of 10 seeds from each treatment were used, with and without surface disinfestation. Surface disinfestation was carried out by immersing the seeds in NaClO (1%)

for three minutes, followed by rinsing with sterilized water and drying at room temperature (25 ± 3 °C).

Using the filter paper method (*Blotter Test*), the seeds were distributed at equal distance apart on three sheets of filter paper previously moistened with distilled water and incubated in 9.0 cm diameter Petri dishes for seven days at 20 ± 2 °C and 12 hours of light. Afterwards, the seeds were analyzed individually under a stereoscopic microscope and the fungi were identified by means of morphological characteristics of their structures. The results were expressed as a percentage of contaminated seeds, for each fungus (BRASIL, 2009).

For seed sanitation, the experiments were evaluated in a completely randomized design, in a 2x6 factorial scheme (surface disinfestation x stages of the conditioning process) and ten replications. The data on fungi incidence found in the seeds (%) was transformed into $(x + 0,01)^{0,5}$ to meet the assumptions of the normality and homogeneity in Shapiro-Wilk tests. Averages of the original data were presented in the tables for a better interpretation of the results.

The data was submitted to analysis of variance, using the F test, and, when significant, the means of the treatments were compared using the Tukey test, at 5% probability. The data about the water content of the seeds before and after accelerated aging was not evaluated statistically.

RESULTS AND DISCUSSION

Regardless of the stage the seed was in the conditioning process or the disinfestation procedure, 13 genera of fungi were detected in the sanitary

analysis of marandu grass seeds: six had a high incidence, with values above of 5%, such as *Phoma* sp., *Alternaria* sp., *Fusarium* sp., *Exserohilum* sp., *Rhizoctonia* sp. and *Curvularia* sp., (Figures 4 and 5).

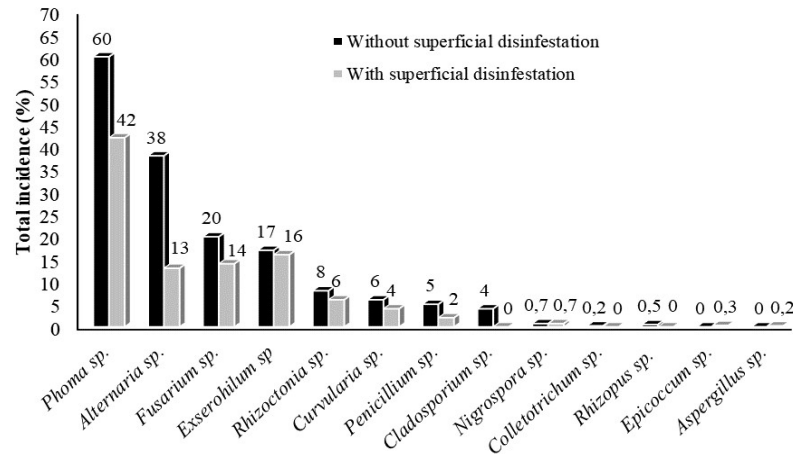


Figure 4. Fungal genera detected, with and without disinfestation, in marandu grass seeds in the conditioning process.



Figure 5. Marandu grass seeds infested by *Phoma* sp., *Alternaria* sp., *Fusarium* sp., *Exserohilum* sp., *Rhizoctonia* sp. and *Curvularia* sp. detected at higher percentage in the seeds during the conditioning process.

Other fungi were detected in low incidence, with values up to 5%, such as *Penicillium*, *Cladosporium*, *Nigrospora*, *Colletotrichum*, *Rhizopus*, *Epicoccum* and *Aspergillus*. *Aspergillus* sp., *Cladosporium* sp., *Curvularia* sp., *Fusarium* sp. and *Phoma* sp. had already been reported in seeds of *B. brizantha* cv. Marandu (MALLMANN et al., 2013). Martins et al. (2017) stated that the occurrence of these phytopathogens did not interfere in the germination of marandu grass seeds.

In contrast, Mentem et al. (1991) and Mallmann et al. (2013) reported that high levels of pathogenic fungi such as *Fusarium* sp. and *Phoma* sp. are of concern since they grow fast and aggressively and can result in seed death even before germination.

Almost all the fungi were found at lower incidence rate after the external disinfestation of the seeds when compared with values before disinfestation, except for *Epicoccum* sp. and *Aspergillus* sp. which were not affected by the disinfestation procedure (Figure 4). The structures of these fungi were probably located within the seeds and the disinfestation with sodium hypochlorite did not influence the microorganisms.

Among the fungi studied, interaction between the conditioning process and the disinfestation procedure was verified only on incidence percentage of *Exserohilum* sp. and *Alternaria* sp. in the seeds (Table 1).

Table 1. Incidence of *Exserohilum* sp. and *Alternaria* sp. in marandu grass seeds with (CD) and without superficial disinfestation (SD) with sodium hypochlorite, according to stages of the conditioning process.

Conditioning process ¹	<i>Exserohilum</i> sp. (%)		<i>Alternaria</i> sp. (%)	
	SD	CD	SD	CD
T1 - Control (unprocessed seeds)	28 bB	11 abA	49 aB	14 abA
T3 - Lower sieve of MAP	21 bB	8 aA	34 aB	10 abA
T6 - Upper discharge of MG I	15 abB	5 aA	35 aA	22 bA
T7 - Intermediate discharge of MG I	21 abA	18 abA	28 aB	10 aA
T8 - Upper discharge of MG II	12 abA	28 bB	29 aA	16 abA
T9 - Intermediate discharge of MG II	7 aA	26 bB	51 aB	8 aA
F Disinfestation (D)	1,15 ^{ns}		101,26 ^{**}	
F Conditioning process (CP)	1,80 ^{ns}		2,25 ^{ns}	
F (DxCP)	8,59 ^{**}		4,14 ^{**}	
C. V. %	55,50		33,28	

** and ^{ns} Significant at 1% and not significant by the test F. Means followed by the same lowercase letter in the column and of the same capital letter in the row, do not differ among themselves by the Tukey test at 5% probability. MAP- air-screen and sieves, MGI- first gravity separator, MGII- second gravity separator.¹ The results of the analysis of T2, T4, T5 and T10 treatments were not presented due to the absence of seeds in the sample.

For *Exserohilum* sp., the stages of the conditioning process, influenced the seeds that had not been subjected to disinfestation. In this case, the seeds from the intermediate discharge of the second gravity separator (T9) presented the lowest incidence of this fungus with values significantly lower than those presented by the seeds from the control (T1) and, even, the intermediate sieve of the air-screen and sieves (T3). However, they did not differ from the other treatments (Table 1).

However, even though there is a lower incidence of *Exserohilum* sp. in this sample, the seeds are not able to be sold or traded, as they did not present the percentage of purity and germination required by the Normative Instruction for the production and commercialization of *B. brizantha* seeds (BRASIL, 2008).

The surface disinfestation significantly reduced the incidence of *Exserohilum* sp. in seeds from the control group (T1), as well as, in those coming from the inferior sieve of the air-screen and sieves (T3) and in the seeds from the top discharge of the first gravity separator (T6). However, in the upper and intermediate discharges from the second gravity separator (T8 and T9), that is, in the last stages of the conditioning process, there was a higher incidence of this fungus in seeds subjected to disinfestation (Table 1). This is possibly due to the presence of spores of the fungus being housed internally in the seeds and when disinfestation was carried out there was a decrease in competition with other fungi on the seed surface and, thus, higher proliferation of *Exserohilum* sp. (QUADROS et al., 2012; AMORIM et al., 2016).

It is worth noting that *Exserohilum* sp. can be transmitted to the seedlings by being present in the seed as reported by Lasca, Vechiato and Kohara (2004) and may cause leaf and stem stains, drying of the leaves and death of the plant (TSUHAKO, 2009). This fungus has been reported in seed lots of marandu grass and xaraes grass from several regions (MARTINS et al., 2017).

The stages of the conditioning process did not influence the incidence of *Alternaria* sp. in the marandu grass seeds, with or without surface disinfestation, as none of the treatments differed from the control. Regarding the disinfestation process, it reduced the incidence of *Alternaria* sp. in the control (T1), intermediary sieves from the air-screen and sieves (T3) and intermediary discharges of the first and second gravity separator (T7 and T9) (Table 1).

Therefore, it was found that a significant percentage of structures of this fungus were lodged superficially on the seeds, and could be disseminated by the machines during the seed conditioning process, through impregnated soil and dust in the equipment and suspended in the environment, causing there not to be a decrease in the incidence of *Alternaria* sp.

Nevertheless, the presence of this pathogen in the seeds was higher than 5% (Table 1 and Figure 4). This would represent a high potential of inoculum, since the genus *Alternaria* is potentially pathogenic among forage grasses and their structures that lodge in inner layers of the seed cannot be eliminated with superficial disinfestation (VECHIATO; APARECIDO; FERNANDES, 2010).

There was no interaction between the stages of seed conditioning and the disinfestation procedure with *Rhizoctonia* sp. and *Curvularia* sp.

in marandu grass seeds (Table 2). Thus, possibly, these fungi were housed both inside and on the surface of the seeds.

Table 2. Incidence of fungi *Rhizoctonia* sp. and *Curvularia* sp. in *Brachiaria brizantha* cv. Marandu seeds according to the stages of the seed conditioning process.

Conditioning process ¹	<i>Rhizoctonia</i> sp. (%)	<i>Curvularia</i> sp. (%)
T1 - Control (unprocessed seeds)	8 ab	6 b
T3 - Lower sieve of MAP	14 b	2 a
T6 - Upper discharge of MG I	5 ab	4 ab
T7 - Intermediate discharge of MG I	7 ab	9 c
T8 - Upper discharge of MG II	5 ab	3 a
T9 - Intermediate discharge of MG II	2 a	8 b
F Disinfestation (D)	4,13*	0,86 ^{ns}
F Conditioning process (CP)	2,82*	2,34*
F (DxE)	1,15 ^{ns}	0,94 ^{ns}
C. V. %	126,33	127,45

* and ^{ns} Significant at 5% and not significant by the test F. Means followed by the same lowercase letter in the column and of the same capital letter in the row, do not differ among themselves by the Tukey test at 5% probability. MAP- air-screen and sieves, MGI- first gravity separator, MGII- second gravity separator.¹ The results of the analysis of T2, T4, T5 and T10 treatments were not presented due to the absence of seeds in the sample.

The percentage incidence of *Rhizoctonia* sp. applied in treatments T3, T6, T7, T8 and T9 did not differ statistically from T1 (Table 2). It is worth noting that any of the evaluated treatments would go to the market with the presence of *Rhizoctonia* sp. This is important mainly because this pathogen, among other fungi, has been reported as responsible for the sudden death of marandu grass pastures, which is one of the main causes of pasture loss in Brazil (MARCHI; FERNANDES; VERZIGNASSI, 2011).

Regarding the effect the stages of the conditioning process have on the incidence of *Curvularia* sp., the highest percentage of contaminated seeds were selected in the intermediate discharge of the first gravity separator (T7). The lowest percentage of seeds with this fungus was obtained in the sample from the intermediate sieve of the air-screen and sieves (T3) and in the upper discharge of the second gravity separator (T8). Therefore, it is possible to infer that the seeds of greater weight had a higher percentage of incidence of this fungus (Tables 1, 2).

The genus *Curvularia* occurs in grasses and has saprophytic behavior, surviving in organic soil matter (SIVANESAN, 1987). Possibly, the seeds of forage grasses that are located at the apex of the panicle were better fed by the plant, well formed, heavier and dispersed first, remaining in contact with the contaminated soil for a longer time until the time of harvest (NERY et al., 2012). Thus, a higher incidence of the pathogen was found in these heavier seeds than the lighter seeds that formed and were dispersed later and thus remain for less time on the soil before being harvested.

CONCLUSION

The seed conditioning process was not able to improve the sanitary quality of the marandu grass seeds.

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RESUMO: A finalidade do beneficiamento de sementes é separar as sementes e suas estruturas associadas em diferentes frações e reter apenas sementes boas e saudáveis. O objetivo deste trabalho foi avaliar o efeito das etapas do beneficiamento sobre a qualidade sanitária de sementes de capim-marandú. As sementes foram amostradas antes e durante o beneficiamento: após a saída da máquina de ar e peneiras (descarga das peneiras superior, intermediária e fundo), primeira mesa gravitacional (deriva ao redor da mesa, descarga superior e intermediária) e segunda mesa gravitacional (descarga superior, intermediária e inferior). A análise sanitária foi realizada pelo método do papel de filtro com e sem desinfestação superficial das sementes, as quais

foram incubadas a 20 ± 2 °C, com fotoperíodo de 12 h durante sete dias. O experimento foi conduzido em delineamento inteiramente casualizado, em esquema fatorial 2x6 (desinfestação superficial x etapas do beneficiamento) e dez repetições. Os dados foram submetidos à análise de variância pelo teste F e quando significativa, as médias dos tratamentos comparadas pelo teste de Tukey, a 5% de probabilidade. Foi possível concluir que o beneficiamento não é capaz de melhorar a qualidade sanitária das sementes de capim-marandú.

PALAVRAS-CHAVE: Blotter test. *Brachiaria brizantha*. Sanidade. Patologia de sementes.

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