Original Research Article

Maximum contact time of fertilizer with Piatã grass seed for implantation of integrated systems

ABSTRACT

Information related to mixing seeds of palisade grass with fertilizers are scarce and contradictory. Goal of this study was to determine the maximum contact time without significant damage on physiological quality of Piatã grass seed mixed with 05-25-15 fertilizer. The trial was carried out in a completely randomized design with six treatments and four replications. Treatments consisted of contact time of the seeds with fertilizer: 0, 24, 48, 72, 96 and 120 hours. Seeds water content was measured and then they were submitted to germination tests, first germination counting, tetrazolium, electrical conductivity, accelerated aging and emergency in sand and, the emergency speed index was calculated. Except for the water content, there was effect of fertilizer contact time with the seeds for all variables. The longer the contact time with the fertilizer, the greater the damage to the physiological quality of Piatã grass seeds. The mixture of Piatã grass seed with 05-25-15 fertilizer must be done for up to 63 hours before sowing.

Keywords: Brachiaria brizantha cv. BRS Piatã, forage crops, plant sciences, planting consortium.

1. INTRODUCTION

Integration between agriculture and livestock is an efficient method for recovering degraded pastures, increase in agricultural income and reduction in greenhouse gas emissions [1, 2]. It improves profitability by assuring cattle performance in the dry season. Besides, it increases biomass cover for proper no-till cropping systems, especially when using grasses as cover crops. MatoGrosso State, in North-Western Brazil, has the largest grain farming and the largest cattle herd in the country.

The most common way to integrate grain crops farming with beef cattle in this area, improving sustainability of local agricultural systems, is a combination as crop sucession of soybeans followed by maize interseeded with palisade grass that can be grazed after maize harvest. The system is locally called "off season bovine", referring to an interseasonal cattle finishing. Grass sowing is carried out just prior, concurrent or subsequent to maize seeding. In the case of simultaneous seeding with maize, forage seed many times is mixed with the fertilizer.

However, seed contact with fertilizers can cause economic losses when maximum contact time limits are not respected. This results in reduced plant stand, leading to lower total

Comment [U1]: germination

Comment [U2]: is

Comment [U3]: The objective of the study was to evaluate

Comment [U4]: Evaluated

Comment [U5]: Germination percentage, first germination counting, viability, eletrical conductivity, accelerated ageing, emergency percentage and emergency speed index were evaluated.

Comment [U6]: Not clear, show how measured parameters (germination percentage, first germination counting, viability, electrical conductivity, accelerated ageing, emergency percentage and emergency speed index) responded to different contact times (0, 24, 48, 72, 96 and 120 hours.) with fertilizer

Comment [U7]: Rewrite: conclusion sentence will adhere to the results (above sentence)

Comment [U8]: Revise recommendation sentence e.g. Piata grass seed could be mixed with fertilizer between 48 to 27 hours before sowing

Comment [U9]: Revise keywords e.g. germination, NPK fertilizer

Comment [U10]: Of forage crops

Comment [U11]: an

Comment [U12]: in particular,

Comment [U13]: space e.g Mato Grosso

Comment [U14]: the

Comment [U15]: succession

Comment [U16]: inter seeded

Comment [U17]: long sentence: replace with short sentences

Comment [U18]: inter seasonal

Comment [U19]: planting

forage dry matter production, geopardizing cattle yields in the dry season and reducing biomass cover for the next soybeans no-till seeding.

Many fertilizers, during their manufacturing process, undergo the action of strong acids. Residual effect of these acids can negatively influence seed's germination and vigor, consequently reducing number of seedlings. Other fertilizers have a high salt content that can damage seeds when in contact. There are also those with high hygroscopicity, absorbing water from ambient, reducing physiological quality of seeds when mixed with them.

A trial carried out bythe authors cited in the reference[3] using the NPK formula 04-14-08, it was found that as contact time of the fertilizer with seeds of *B. brizantha* cv. Marandu increased germination and vigor were reduced. The authors emphasize that the salt on potassium chloride can influence seeds' water content, resulting in poorer germination as well as it can break seeds' tegument, resulting in increased electrical conductivity. That study recommended a mixture should be held for a maximum of 12 hours to obtain better results at sowing. The authors cited in the reference [4] argue that emergency of *B. brizantha*seedlings is not affected since the mixture of seeds with phosphate fertilizers and formulations to not exceed 96 hours prior to sowing.

Given this diversity of results, goal of this study was to determine the maximum contact time without significant damage on physiological quality of Piatã grass seed mixed with 05-25-15 fertilizer.

2. MATERIAL AND METHODS

The experiment was carried out in the Seed Laboratory at the Federal University of MatoGrosso in 2015. The trial was carried out in a completely randomized design with six treatments and four repetitions. Treatments were contact times of Piatã grass seed (*Brachiariabrizantha*(syn. *Urochloa*) cv. Piatã BRS) with the fertilizer: 0, 24, 48, 72, 96 and 120 hours. Fertilizer used was the formulation 05-25-15, commonly used for maize, composed by monoammonium phosphate, superphosphate, triple superphosphate and potassium chloride.

To adjust the ratio between seeds and fertilizer, it was necessary to establish the cultural value of the given seeds batch, sowing rate and amount of fertilizer. To determine the cultural value (CV), purity and viability tests were carried out. Usually, germination is used to determine cultural value of seeds, however, this study used the viable seeds test, since it became the most commonly used analysis in the forage seeds market after the Brazilian regulation Normative Instruction nº 30 [5] was published.

In the purity test, two 5.0 g sub-samples were used, separating Piatã grass seeds from other seeds as well as inert particles [6], using sieve and clamp. Subsequently, fractions were weighed and results added together and compared to the initial mass, following the tolerance required by the Brazilian Rules for Seed Analysis [6]. Proportion of pure seed was expressed as percentage.

The lot of Piatã grass seeds used was in the study had 75% purity and 80% viability by the tetrazolium test, following the methodology from from Brazilian Rules for Seed Analysis [6], resulting in cultural value of 60%. Thus, the fertilizer/seed rate used was 60:1. The mixtures were placed in plastic bags for time periods pre-set for each treatment. Seeds from the control treatment (time zero) had no contact with the fertilizer. At the schedule times manual separation of seeds was carried out using sieves and clamps. Seeds water content was

Comment [U20]: jeopardising

Comment [U21]: The residual

Comment [U22]: vigour

Comment [U23]: the number

Comment [U24]: hygroscopic

Comment [U25]: the physiological

Comment [U26]: by the

Comment [U27]: reduce space

Comment [U28]: integument

Comment [U29]: space

Comment [U30]: delete

Comment [U31]: The objective of this study was to evaluate

Comment [U32]: To the physiological

Comment [U33]: Space: Mato Grosso

Comment [U34]: evaluated

Comment [U35]: Mono-ammonium

Comment [U36]: Rewrite: do not start sentence with 'to'
e.g. It was necessary to establish the cultural value of the given seeds batch, sowing rate and amount of fertlizer to adjust the ratio between seeds and fertilizer.

Comment [U37]: Similar comment: do not start sentence with 'to'

Comment [U38]: Long sentence: this phrase makes your wrting more difficult to understand. Revise the sentence, e.g. Very often, germination test is used to determine cultural value of seeds. However, the viable seed test is used in this study because is the most common test used to analyse forage seeds for the market. This also adhere to the Brazilian regulation Normative instruction no 30 [5].

Comment [U39]: The proportion

Comment [U40]: a percentage

Comment [U41]: delete

Comment [U42]: Complex sentence: revise and make it simple sentence

Comment [U43]: Revise the sentence

Comment [U44]: scheduled times, the

measured and the following tests were carried out: germination, first germination counting, tetrazolium, electrical conductivity, accelerated aging and emergency in sand. Finally emergency speed index was calculated.

For determining water content, three samples of 4.0 g each were placed in oven for 24 hours at 105±1°C temperature. After drying, samples were cooled down and weighted with analytical scale [6].

The methodology used for the standard germination test is described in Brazilian Rules for Seed Analysis[6], using four replicates of 50 seeds for each treatment, i.e, contact time of seeds with the fertilizer. Seeds were equidistantly laid in "gerbox" type germination boxes over two sheets of blotting paper substrate moistened with distilled water at the rate of two and half times the weight of the dry paper. Then, the boxes were taken to BOD germinating chamber with adjustable photoperiod of 12 hours and alternating temperature 35/20°C (12 hours in light at 35°C and 12 hours in absence of light at 20°C). First counting was carried out on the seventh day, considering as germinated the seeds with at least 1mm primary root. On the 21st day the last counting was carried out to determine the percentage of germination.

Seed viability was determined by the tetrazolium test using four replications of 50 seeds each treatment. Seeds were pre-moistened between germination paper sheets and placed in growth chamber for 18 hours without light at temperature of 30°C. Subsequently, they were cut longitudinally to expose the embryo and only one of the seeds' parts was put in contact with the tetrazolium salt solution (2, 3, 5 triphenyltetrazolium chloride) at 0.5%, and placed in BOD for 3 hours to dye seeds having living tissue. Next, seeds were washed and read was carried out according to RAS [6], classifying them into viable and non-viable.

Electric conductivity was measured as described in AOSA [7]. Four samples of 50 seeds were use, which were weighed on analytical balance and placed in a plastic container with 75 mL of distilled water and then brought to the growth chamber where they remained for a period of 24 hours at 25°C. Afterwards, exudates released were measurude using a conductivity meter. Results were expressed in $\mu S\ cm^{-1}\ g^{-1}$.

For the accelerated aging test, the methodology proposed bythe authors cited in the reference[8] was followed, where seeds were distributed on aluminum screen attached to "gerbox" type boxes with 40 mL of distilled water at the bottom. Afterwards boxes were covered and placed in a growth chamber for a period of 36 hours at 42 °C. Standard germination test was carried out reading the germinated seeds after seven days.

For the emerging test, seeds were sown in trays under light incidence for 12 hours, using 50 seeds per repetition. In each tray were placed 2.5 kg of washed sand, screened and sterilized at 105±1 °C for 24 hours. Seeds were placed in sand at a depth of 1 cm (Brasil, 2009), and the humidity kept around 60% of field capacity. During 21 days, i.e, until emerging stabilization, a daily counting was carried out, considering emerged the seedlings 1 mm high above substrate level. The daily count of seedlings was necessary to determine the emergency speed index (ESI) calculated according to the methodology described by the authors cited in the reference [9].

Data were submitted to analysis of variance and regression analysis at 5% probability.

Comment [U45]: the sand

Comment [U46]: Finally,

Comment [U47]: Complex sentence: revise; e.g. Three samples of 4 g were placed in an oven for 24 hours at 105±1°C temperature to determine water content

Comment [U48]: An oven

Comment [U49]: an analytical

Comment [U50]: i.e.

Comment [U51]: day,

Comment [U52]: the growth

Comment [U53]: a temperature

Comment [U54]: long sentence, revise and make simple short sentences

Comment [U55]: Revise: What RAS stands for? In the reference list I noted Rules for seed analysis (RSA)

Comment [U56]: used

Comment [U57]: was

Comment [U58]: ml

Comment [U59]: revise long sentence and make simple short sentences

Comment [U60]: measured

Comment [U61]: by the

Comment [U62]: revise the sentence: e.g. do not start sentence with 'for' follow mentioned examples

Comment [U63]: afterwards,

Comment [U64]: revise the sentence: e.g. do not start sentence with 'for' follow mentioned examples

Comment [U65]: the sand

Comment [U66]: Revise: is this the reference? If it is the reference the corect reference is [9] or [6]. Note check your reference list

Comment [U67]: i.e.

Comment [U68]: delete

Comment [U69]: revise e.g. other studies

Comment [U70]: was

Comment [U71]: revise data analysis; replace the statement with a detailed sentences showing statistical packages used to run ANOVA and regression analysis

3. RESULTS AND DISCUSSION

There was a negative linear effect between contact time of seeds with the fertilizer for all variables, except for the electrical conductivity, which was described by positive linear model, and the water content, which did not vary.

Regardless of contact time with the fertilizer, seeds water content was in average 10.09%. The absence of alteration in the water content of the seeds can come from the storage of the seeds in semipermeable packaging, making it difficult to absorb moisture from the air. In addition, the chemical composition of the seeds, influenced by genetics, environmental conditions and plant traits [10], may alter the hygroscopicity of the seeds, and fatty acids have a hydrophobic characteristic, and with an antagonistic relation to the content of protein [11].

With opposite results, the authors cited in the reference [12] observed a positive linear effect for water content in *Brachiariaruziziensis*seeds in contact with urea. The authors attributed this effect to the high hygroscopicity of the fertilizer, which could have transferred excess water over to the seeds. In the case of this work, NPK formulation 05-25-15 contains no urea.

There was a reduction of 36.4; 36.7; and 25.0% in the germination rate, first count and viability of Piatã grass seeds having contact time of 120 hours when comparing to seeds that had no contact with the fertilizer (Figure 1).

Comment [U72]: revise results senstence

Comment [U73]: Figure 1

Comment [U74]: revise the sentence

Comment [U75]: hygroscopic

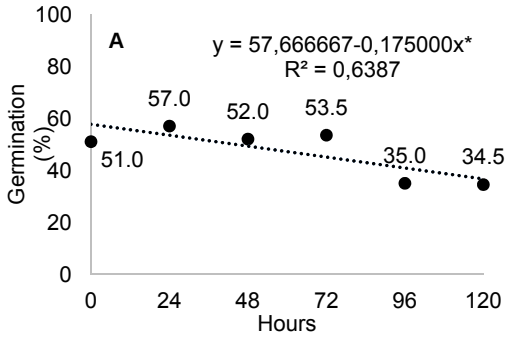
Comment [U76]: revise: replace translation with proper phrase

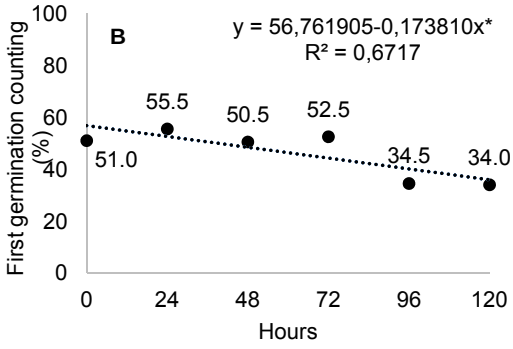
Comment [U77]: space

Comment [U78]: hygroscopic

Comment [U79]: a contact

Comment [U80]: Revise complex sentence





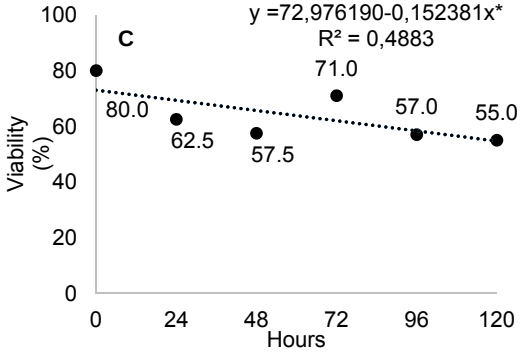


Figure 1. Germination percentage (A), first count of germination (B) and viability (C) of *Brachiariabrizantha* cv. Piatã seeds according to time of contact with the fertilizer NPK 05-25-15.

Probably due to tegument rupture, embryo exposure and electrolyte leakage (confirmed by the results of the electrical conductivity test), caused by factors intrinsic to the fertilizer, with acid pH and salinity index high. Moreover, the rapid absorption of water by the seed during

Comment [U81]: the time

Comment [U82]: 05:25:15

Comment [U83]: integument

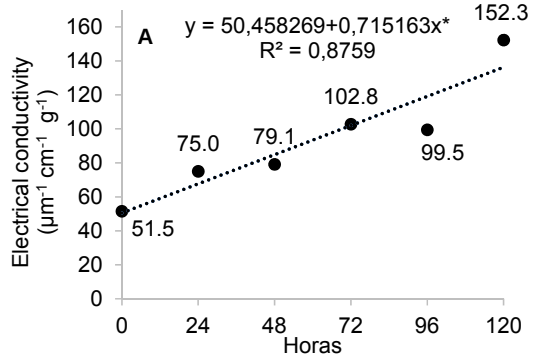
Comment [U84]: complex sentence; revise; follow mentioned examples

the imbibition phase may influence the germination and viability process, as it causes damage to the seeds' tissues [13, 14].

Similar results were found by the authors cited in the reference [15], who tested seed contact time of *Brachiariabrizantha* with triple superphosphate.

In this work, germination percentages obtained in the standard germination test were lower than the results from the tetrazolium test (Figures 1A and 1C), what shows that part of viable seeds did not germinate. This may be caused by pathogen action or physiological dormancy that occurs in *Brachiaria* seeds, as observed by the authors cited in the reference [16, 17]. According to InstruçãoNormativa nº 30 (Brasil, 2008; 2010), for selling *Brachiariabrizantha* seeds, it is necessary germination or viability of 60%; therefore, this batch has reached market standard only through the analysis of viability.

The higher the seed contact time with fertilizer, the higher were electrical conductivity values (Figure 2A) with 170.0% increase when comparing 120 hours contact with no contact. This increase was expected, since the fertilizer has acid residues and high salinity, what can damage seed integument, releasing electrolytes, increasing levels of electrical conductivity.



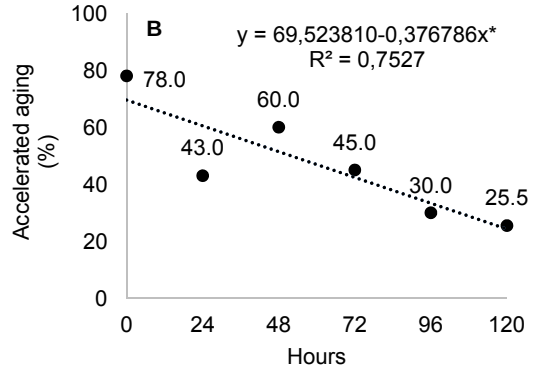


Figure 2. Electrical conductivity (A) and accelerated aging (B) of *Brachiariabrizantha* cv. Piatã seeds according to time of contact with the fertilizer NPK 05-25-15.

Comment [U85]: revise; delete in this work

Comment [U86]: which

Comment [U87]: revise: phrase must be similar to the one mentioned earliar

Comment [U88]: Revise: Brazil or Brasil

Comment [U89]: Revise: correct is [18]; [19] to replace (Brasil 2008;2018)
Note: Correct Reference Numbering

Comment [U90]: which

Comment [U91]: the time

Comment [U92]: 05:25:15

The authors cited in the reference[18]evaluated *Brachiariabrizantha* seeds in contact with potassium chloride only and found that there was a positive linear effect on values of electrical conductivity. According to the authors, this increase is justified by the high salt content of the fertilizer, which caused an increase in electrical conductivity rate up to 30 times higher than the values found in the control treatment, due to disruption of seed integument and release of electrolytes, as well as to potassium chloride residue in the seeds.

Comment [U93]: revise e.g. the study evaluated....
place [18] at the end of the sentence

Comment [U94]: Long sentence: revise & make short sentence

The authors cited in the reference [19] analyzing seeds of *Brachiariabrizantha* cv. Marandu in contact with granulated single superphosphate, powdered single superphosphate and granulated monoammonium phosphate also observed a positive linear increase in electrical conductivity values when seeds remained in touch with the single superphosphate, either granulated or powdered.

Comment [U95]: Revise: similar comment to above paragraph

Comment [U96]: analyse

Comment [U97]: mono-ammonium

As contact time of Piatã grass seeds with the fertilizer increased, there was a 65.0% reduction in germination in those submitted to the accelerated aging test when comparing contact time of 120 hours with no contact (Figure 2B). This shows reduction in seed vigor by increasing seeds contact time with the fertilizer. However, for seeds that had no contact with the fertilizer (time zero), there was higher percentage of germination in the accelerated aging test (69.5%) than in the standard germination test (57.7%), what was probably caused by breaking physiological dormancy or reducing pathogens level in seeds due to high temperatures in the accelerated aging test.

Comment [U98]: a reduction

Comment [U99]: vigour

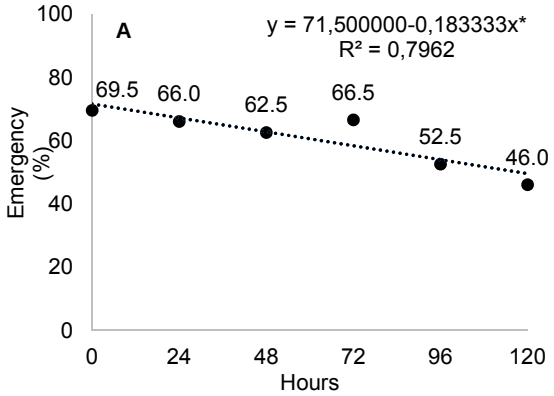
Comment [U100]: revise long sentence

The authors cited in the reference [20] analyzing seeds of *Brachiariabrizantha* cv. MG-5 Vitória, concluded that accelerated aging is a method capable of overcoming the physiological seeds dormancy.

Comment [U101]: Revise & check mentioned comments

As for the emergency of Piatã grass seedlings, there was a 30.8% decrease when comparing 120 hours contact time with no contact (Figure 3A). When comparing these results with those obtained in standard germination test (Figure 1A), there is a higher percentage of emergency, which indicates that the seed in the sand in emergency test was better able to express their productive potential, as the values were closer to those in the viability test (Figure 1C).

Comment [U102]: the emergence



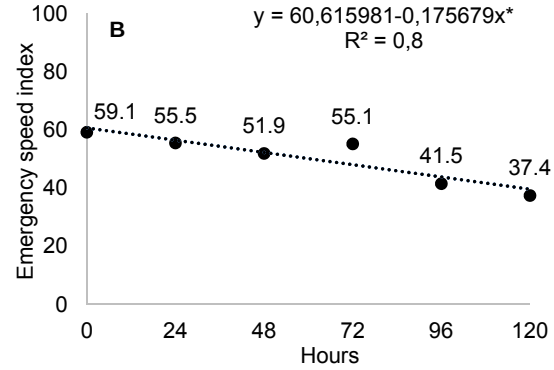


Figure3. Emergency percentage (A) and emergency speed index (B) of *Brachiariabrizantha* cv. Piatã seeds according to time of contact with the fertilizer NPK 05-25-15.

This effect may be due to the increase of seed contact surface with sand or by different conditions for the development of pathogens present in seed surface. The authors cited in the reference [18] evaluating *Brachiariabrizantha* seeds in contact with potassium chloride, obtained results similar to those found in this work, in which some contact times in the emergency test exceeded the germination values.

Like results for emergency rates, values found for emergency speed index (EVI) decreased when seed contact time with the fertilizer was increased, following a negative linear pattern, with a 34.8% decrease when comparing 120 hours contact time with the control (Figure 3B). The authors cited in the reference [21] found similar results when testing *Brachiariabrizantha* seed contact time with the NPK formulation 04-14-08.

Considering that for the marketing *Brachiariabrizantha* seeds it is accepted a minimum germination or viability of 60% [22, 23], standard was kept by the viability test until 85 hours after mixing the seeds with the NPK fertilizer 05-25-15.

Comment [U103]: the

Comment [U104]: 05:25:15

Comment [U105]: Revise complex sentence, follow comments mentioned earlier

Comment [U106]: Revise or delete

Comment [U107]: Revise e,g considering marketing of *Brachiariabrizantha* seeds

Comment [U108]: the standard

Comment [U109]: 05:25:15

Regarding germination, using the standard germination test (on paper method) it was observed a result below the minimum standard required for marketing. However, adopting the emergency test on sand as germination test, what is possible, as described in the RAS [6], the standard germination levels required on InstruçãoNormativa nº 30 [22] are kept, when Piatã grass seeds are left in contact with the fertilizer for no more than 63 hours. The authors cited in the reference [4] evaluating *Brachiariabrizantha* seeds in contact with phosphate fertilizers, potassium chloride and powdered formulations observed for all studied fertilizers, a negative effect on seeds at a 96-hour contact time.

Therefore, for large farms, or to speed up seeding logistics, Piatã grass seeds can be mixed with the NPK formulation 05-25-15 up to 63 hours before sowing, keeping acceptable seeds viability and seedling emergence (60%) [22, 23], can origin implantation of integration between agriculture and livestock, with adequate stand of grass seedling in area.

Comment [U110]: Revise: check earlier comments

Comment [U111]: Complex & long sentence; check earlier comments

Comment [U112]: Revise the compex sentence

Comment [U113]: Revise; e.g It was found that in large farms, or to speed up seeding logistics, Piatã grass seeds can be mixed ..

Comment [U114]: Revise: not clear

4. CONCLUSION

It is recommended simultaneous sowing of grass Piatã up to 63 hours after mixing the seeds with the fertilizer NPK 05-25-15.

Comment [U115]: Revise: write the detailed conclusion phrases based on the findings/ results. Recommedation sentences will follow after correctly experesing the results

COMPETING INTERESTS

We declare that no competing interests exist.

REFERENCES

Comment [U116]: Revise reference lists following numbering changes in the materials and methods, and results and discussion.

1. Balbino Junior AA, Moraes A, Veiga M, Pelissar A, Dieckow J. Crop-livestock integration: intensification of the use of agricultural areas. English. Rural Science. 2009; 39 (6): 1925-1933. DOI: 10.1590 / S0103-84782009005000107

2. Vilela L, Martha Junior GB, Macedo MCM, Marchão R L, Guimarães Júnior R., Pulrolnik, K et al. Integration systems for livestock farming in the Cerrado region. Agricultural Research. 2011; 46 (10): 1127-1138. DOI: 10.1590 / S0100-204X2011001000003

3. Lima EDV, Tavares JC, Azevedo VR, Leitão-Lima PS. Mixture of Brachiariabrizanth seeds with NPK fertilizer. Rural Science. 2010; 40 (2): 471-474. DOI: 10.1590 / S0103-84782010005000003

4. Mateus GP, Borghi E, Marques RR, Bôas RLV, Crusciol CAC. Fertilizer sources and contact periods and seed germination of Brachiariabrizantha. Brazilian Journal of Soil Science. 2007; 31 (1): 177-183. English. DOI: 10.1590 / S0100-06832007000100018

277

278 5. Brazil. Ministry of Agriculture. Normative Instruction No. 30 of October 26, 2010.
279 Amendment of Normative Instruction No. 30 of May 21, 2008. Accessed 02 Jan 2019.
280 Available: www.agricultura.gov.br
281
282 6. Brazil. Ministry of Agriculture, Livestock and Supply. Rules for seed analysis.Brasília:
283 MAPA / ACS; 2009.
284 7.AOSA. Association of Official Seed Analysts. Seed Vigour Testing Handbook.Washington:
285 AOSA; 1983.
286
287 8. Marks Filho J. Accelerated aging test. In: Krzyzanowski FC, Vieira RD, France Neto JB.
288 Seed vigor: concepts and tests. Londrina: Abrates; 1999.
289
290 9. Maguire LD. Speed of germination aid in selection and evolution forseedling emergence
291 and vigor. Crop Science. 1962, 2: 176-177. DOI: 10.2135 /
292 cropsci1962.0011183X000200020033x
293
294 10.MTCS boards, Pôrto NA, Canuto MFS, Bruno RLA. Chemical Composition of ManihotMill
295 Species Seeds. (Euphorbiaceae). Brazilian Journal of Biosciences. 2007; 5 (1): 621-623.
296 English.
297
298 11. Delarmelino-Ferraresi LM, Villela FA, Aumonde TZ. Physiological performance and
299 chemical composition of soybean seeds. Brazilian Journal of Agricultural Sciences. 2014; 9
300 (1): 14-18. English. DOI: 10.5039 / agraria.v9i1a2864
301
302 12. Dan HA, Dan LGM, Barroso ALL, Braccini AL, Puccinin GG. Mixture of seeds of
303 BrachiariaruziziensisG.et E with urea aiming at the implantation of the crop-livestock
304 integration system. Revista Caatinga.2011; 24 (4): 68-73. English.
305
306 13. Derré LO, Custodio CC, Agostini EAT, WEX War. Obtaining the embedding curves of
307 coated and uncoated seeds of Urochloabrizanthae Urochloa ruziziensis.
308 ColloquiumAgrariae. 2013; 9 (2): 103-111. English. DOI: 10.5747 / ca.2013.v09.n2.a094
309
310 14. Toledo LF, Carvalho-E-Silva SP, Sánchez C, Almeida MA, Haddad CFB. The review of
311 the Brazilian Forest Act: harmful effects on amphibian conservation. Biota Neotropica.2010:
312 10 (4): 35-38. DOI: 10.1590 / S1676-06032010000400003
313
314 15. Lima EDV, Tavares JC, Silva EC, Leitão-Lima P. Triple superphosphate as a way of
315 distribution of Brachiariabrizantha seeds for pasture renewal in Amazonia. Revista Brasileira
316 de Zootecnia.2009; 38 (5): 796-800. English. DOI: 10.1590 / S1516-35982009000500003
317
318 16. Dias MCLL, Alves SJ. Evaluation of the viability of Brachiariabrizantha (Hochst. Ex A.
319 Rich) Stapf seeds by the tetrazolium test. Brazilian Journal of Seeds. 2008; 30 (3): 145-151.
320 English. DOI: 10.1590 / S0101-31222008000300019
321
322 17. Pariz CM, Ferreira RL, Sá ME, Andreotti M, Chioderoli CA, Ribeiro AP. Physiological
323 quality of Brachiaria seeds and evaluation of dry mass yield in different systems of crop-
324 livestock integration under irrigation. Tropical Agriculture Research. 2010; 40 (3): 330-340.
325 DOI: 10.5216 / pat.v40i3.6590
326

Comment [U117]: Revise ; follow journal quidelines

18. Lima EV, Tavares JCS, Leitão-Lima PS, Pinheiro DP. Contact periods of the KCl fertilizer on the physiological quality of BrachiariabrizanthaStapf seeds. Amazon: Science & Development. 2013; 8: 53-64. English.

19. Peres AR, Vazquez GH, Cardoso RD. Physiological potential of Brachiariabrizantha cv. Maranduseedskept in contactwithphosphaticfertilizers. Brazilian Journal of Seeds. 2012; 34 (3): 424-432. DOI: 10.1590 / S0101-31222012000300009

20. Cardoso ED, Sá ME, Haga KI, Binotti FFS, Nogueira DC, Valério Filho WV. Physiological performance and dormancy overcoming of Brachiariabrizantha seeds submitted to chemical treatment and artificial aging. Semina: Agrarian Sciences. 2014; 35 (1): 21-38. DOI: 10.5433 / 1679-0359.2014v35n1p21

21. Lima EDV, Tavares JC, Azevedo VR, Leitão-Lima PS. Mixture of Brachiariabrizanth seeds with NPK fertilizer. Rural Science.2010; 40 (2): 471-474. DOI: 10.1590 / S0103-84782010005000003

22. Brazil. Ministry of Agriculture, Livestock and Supply. Normative Instruction No. 30 of May 21, 2008. Norms and standards for production and commercialization of seeds of tropical forage species. 2008. Accessed 15 Feb 2019.
Available: www.adagri.ce.gov.br

23. Brazil. Ministry of Agriculture. Normative Instruction No. 30 of October 26, 2010. Amendment of Normative Instruction No. 30 of May 21, 2008. Accessed 16 Feb 2019.
Available: www.adagri.ce.gov.br

Comment [U118]: Well written in the reference & adopt the same words in the context (Materials & Mothods and Results & Discussion)