

**Maximum contact time of “Piatã grass” *Brachiaria brizantha* (Poaceae) seeds with fertilizer for germination**

**ABSTRACT**

Data regarding the mixing of palisade grass seeds with fertilizers are scarce and contradictory. The aim of this study was to evaluate the maximum contact time without significant damage on physiological quality of Piatã grass seeds when mixed with 05:25:15 NPK fertilizer. The experiment was conducted in a completely randomized design with six treatments and four replications. Treatments consisted of six contact times of the seeds with the fertilizer: 0, 24, 48, 72, 96 and 120 hours. The variables following variables were evaluated: water content, germination percentage, first germination count, seed viability, electrical conductivity, accelerated aging, emergence percentage and emergence speed index. Except for the water content, there was an effect of the fertilizer contact times with the seeds over all variables. There was a decreasing linear effect of the contact time of the seeds with the fertilizer for all variables, except for electrical conductivity. The average water content of 10.09% was observed. After 120 hours of the fertilizer/seeds mixture, a reduction of 36.4% was observed in germination, of 36.7% in the first germination count, of 25.0% in seed viability, and of 65.0% in the germination of seeds subjected to the accelerated aging. There was an increase of 170.0% in the electrical conductivity of the Piatã grass seeds. The minimum standard of 60% germination, viability or emergence, required for the commercialization of forage seeds, should be maintained to ensure the ideal forage population in the establishment of the crop. Based on the emergence and viability tests, the commercialization levels can be maintained by performing the fertilizer/seeds mixture up to 63 hours before sowing. After 63 hours of seed and fertilizer mixing, a reduction of the initial vigor of the seeds can be observed, although not compromising the crop establishment or causing degradation of soil. Therefore, Piatã grass seeds can be mixed with the 05:25:15 NPK fertilizer up to 63 hours before sowing.

**Keywords:** *Brachiaria brizantha* cv. Piatã, germination, NPK fertilizer, grass seeds.

**1. INTRODUCTION**

Integration of forage crops and livestock is an efficient method for recovering degraded pastures, increasing agricultural income and reducing greenhouse gas emissions [1, 2]. It improves profitability by ensuring cattle performance in the dry season. Besides, it increases biomass cover for proper no-till farming systems, particularly when using grasses as cover crops. Mato Grosso State, in northwestern 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 is the crop succession of soybean followed by an interseeding of maize with 'palisade grass' [*Brachiaria brizantha* (Hochst. ex A. Rich.) Stapf, Poaceae]. This system is locally called 'bovine off-season', referring to an interseasonal cattle finishing. Grass sowing is performed just prior, concurrent or subsequent to maize sowing. In the case of simultaneous seeding with maize, forage seeds are often mixed with the fertilizer.

However, seed contact with fertilizers might cause economic losses when maximum contact time limits are not observed, resulting in reduced plant stands and lower production of total forage dry matter, thus jeopardizing cattle yield in the dry season and reducing biomass cover for the next no-till soybean sowing.

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

In a trial performed by the authors cited in the reference [3] using the 04-14-08 NPK formula, they verified that the increase in the contact time of the fertilizer with seeds of *B. brizantha* cv. Marandu compromised germination and vigor. The authors emphasize that the salt in the potassium chloride can influence the water content of seeds, resulting in poorer germination for its effect in disrupting the seed integument and increasing electrical conductivity. That research recommended that the fertilizer/seeds mixture should be held for a maximum of 12 hours to obtain better results at sowing. The emergence of *B. brizantha* seedlings is not affected as long as the mixture of seeds with phosphate fertilizers and formulations does not exceed 96 hours prior to sowing [4].

The aim of this study was to evaluate the maximum contact time of *B. brizantha* cv. BRS Piatã seeds mixed with 05:25:15 fertilizer without presenting significant damage to the physiological quality of the seeds.

## 2. MATERIAL AND METHODS

The experiment was conducted in the Seed Laboratory of the Federal University of Mato Grosso (UFMT), in 2015. The trial was performed in a completely randomized design with six treatments and four replications. Treatments consisted of six contact times of Piatã grass seeds (*Brachiaria* (syn. *Urochloa*) *brizantha* cv. Piatã) with the fertilizer: 0, 24, 48, 72, 96 and 120 hours. The utilized fertilizer was the 05:25:15 NPK formulation, commonly used for maize, composed of mono-ammonium phosphate, superphosphate, triple superphosphate, and potassium chloride.

The seed batch cultural value, sowing rate and fertilizer amount were determined in order to adjust the ratio between seeds and fertilizer. Cultural value (CV), purity and viability tests were performed. Germination tests are often employed to determine the cultural value of seeds; however, the tetrazolium test for seed viability was employed in this study for being the most common test for forage seeds analysis, also adhering to the Normative Instruction no 30 [5] in Brazilian regulation.

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, the fractions were weighed and the results combined and compared with the initial mass, following the

72 tolerance required by the Brazilian Rules for Seed Analysis [6]. The proportion of pure seeds  
73 was expressed as a percentage.

74 The batch of Piatã grass seeds utilized in the study had 75% purity and 80% viability,  
75 resulting in a cultural value of 60%; therefore, the fertilizer/seeds rate was 60:1. The  
76 fertilizer/seeds mixtures were stored in plastic bags for five time periods: 24, 48, 72, 96 and  
77 120 hours. Seeds from the control treatment (time zero) had no contact with the fertilizer. In  
78 the scheduled times, the manual separation of seeds was performed using sieves and  
79 clamps. The water content of the seeds was measured and the following tests were  
80 performed: germination, first germination count, tetrazolium test for seed viability, electrical  
81 conductivity, accelerated aging and seedling emergence in sand. Afterward, the emergency  
82 speed index was calculated.

83 Three 4 g samples were placed in a forced air drying oven for 24 hours at  $105\pm 1^{\circ}\text{C}$  to  
84 determine the water content. After drying, the samples were cooled and subsequently  
85 weighed with an analytical balance [6].

86 The methodology used for the standard germination test is described in the Brazilian Rules  
87 for Seed Analysis [6], using four replicates of 50 seeds for each treatment. Seeds were  
88 equidistantly laid in crystal polystyrene germination boxes (gerbox) over two sheets of  
89 blotting paper substrate moistened with distilled water at the rate of two and a half times the  
90 weight of the dry paper. Afterward, the boxes were taken to a BOD (Biochemical Oxygen  
91 Demand) germinating chamber with an adjustable photoperiod of 12 hours and alternating  
92 temperature of  $35/20^{\circ}\text{C}$  (12 hours in light at  $35^{\circ}\text{C}$  and 12 hours in absence of light at  $20^{\circ}\text{C}$ ).  
93 The first count was performed on the seventh day, considering as germinated the seeds with  
94 at least 1mm of the primary root. On the 21st day, the last count was performed to determine  
95 the germination percentage.

96 Seed viability was determined by the tetrazolium test using four replications of 50 seeds per  
97 treatment. Seeds were pre-moistened between germination paper sheets and placed in the  
98 BOD chamber for 18 hours, without light, at a temperature of  $30^{\circ}\text{C}$ . Subsequently, they were  
99 longitudinally cut to expose the embryo. Only one of the seed halves was put in contact with  
100 the tetrazolium salt solution (2, 3, 5 triphenyltetrazolium chloride) at 0.5%, being later placed  
101 in the BOD for 3 hours to dye the living tissues. The seeds were then washed and the  
102 viability evaluation was performed according to the methodology described in reference  
103 works [6], classifying them as viable or non-viable.

104 The electrical conductivity was measured as described in AOSA [7]. Four samples of 50  
105 seeds were used. The seeds were weighed on an analytical balance and placed in a plastic  
106 container with 75 ml of distilled water, then taken to the BOD chamber where they remained  
107 for a period of 24 hours at  $25^{\circ}\text{C}$ . Afterward, released exudates were measured using a  
108 conductivity meter. Results were expressed in  $\mu\text{S cm}^{-1} \text{ g}^{-1}$ .

109 The methodology proposed by other studies [8] was followed for the accelerated aging test,  
110 where seeds were distributed on an aluminum screen attached to the 'gerbox' type boxes  
111 containing 40 ml of distilled water at the bottom. The boxes were then covered and placed in  
112 the BOD growth chamber for a period of 36 hours at  $42^{\circ}\text{C}$ . The standard germination test  
113 was performed to evaluate the germinated seeds after seven days.

114 The seeds were sown in trays under 12-hour light incidence, using 50 seeds per replicate in  
115 the emergence test. In each tray were placed 2.5 kg of washed sand previously screened  
116 and sterilized at  $105\pm 1^{\circ}\text{C}$  for 24 hours. Seeds were placed at a 1 cm depth [6], and the  
117 humidity was kept around 60% of field capacity. A daily count was performed for 21 days

118 until emergence stabilization, considering emerged the seedlings with 1 mm above the  
119 substrate level. The daily seedling count was necessary to determine the emergence speed  
120 index (ESI), calculated according to the methodology described by other studies [9].

121 The analysis of variance (ANOVA) considered the effect of the seeds/fertilizer contact time in  
122 the forage species (*B. brizantha* cv. Piatã). A 5% probability level was considered. The  
123 ANOVA presented a significant difference, and therefore linear regression analysis was  
124 performed. A 5% probability level was considered. The SANEST computer software was  
125 utilized for the analyses [10].

126

### 127 3. RESULTS AND DISCUSSION

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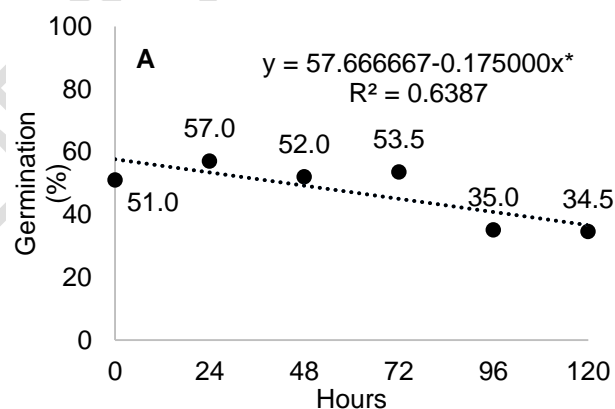
129 There was a decreasing linear effect of the seeds contact time with the fertilizer for all  
130 variables, except for the water content and the electrical conductivity (Fig. 1, 2 and 3).

131 No effect of the contact time on the water content of the seeds was verified, with an average  
132 water content of 10.09% being observed.

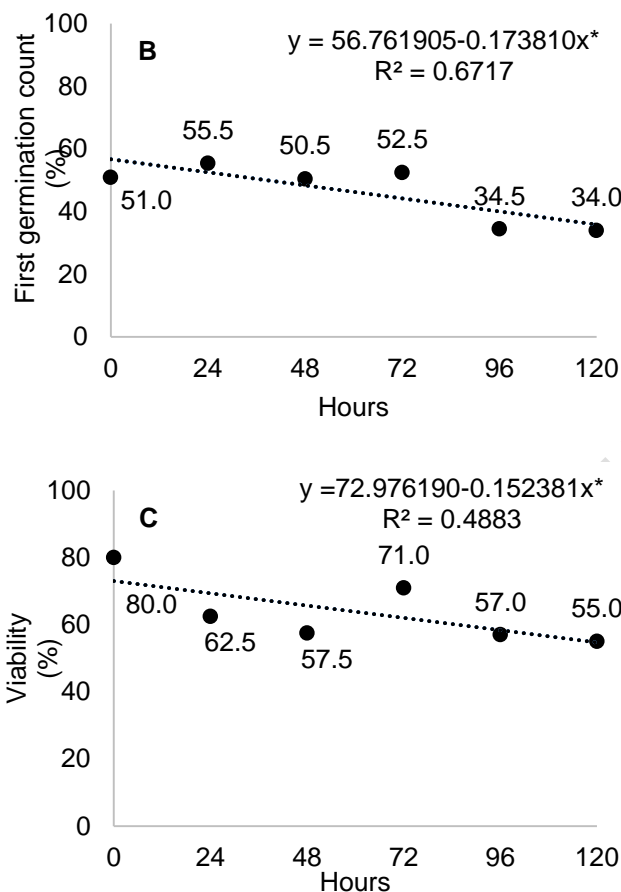
133 The absence of alteration in the water content of the seeds might come from the storing in  
134 semi-permeable packaging, making it difficult to absorb moisture from the air. In addition, the  
135 chemical composition of the seeds, influenced by genetics, environmental conditions and  
136 plant traits [11], may alter the hygroscopicity of the seeds. Furthermore, fatty acids present a  
137 hydrophobic characteristic, with antagonistic relation to the protein content [12].

138 The seeds of *Brachiaria ruziziensis* presented a positive linear effect for the water content  
139 when in contact with urea, with this effect being attributed to the high hygroscopicity of the  
140 fertilizer, which could have transferred the excess of water to the seeds. In the case of  
141 present research, the NPK formulation 05:25:15 contains no urea [13].

142 After 120 hours of mixture, there was a reduction of 36.4% in germination, of 36.7% in first  
143 germination count and of 25.0% in the viability of Piatã grass seeds (Fig. 1).



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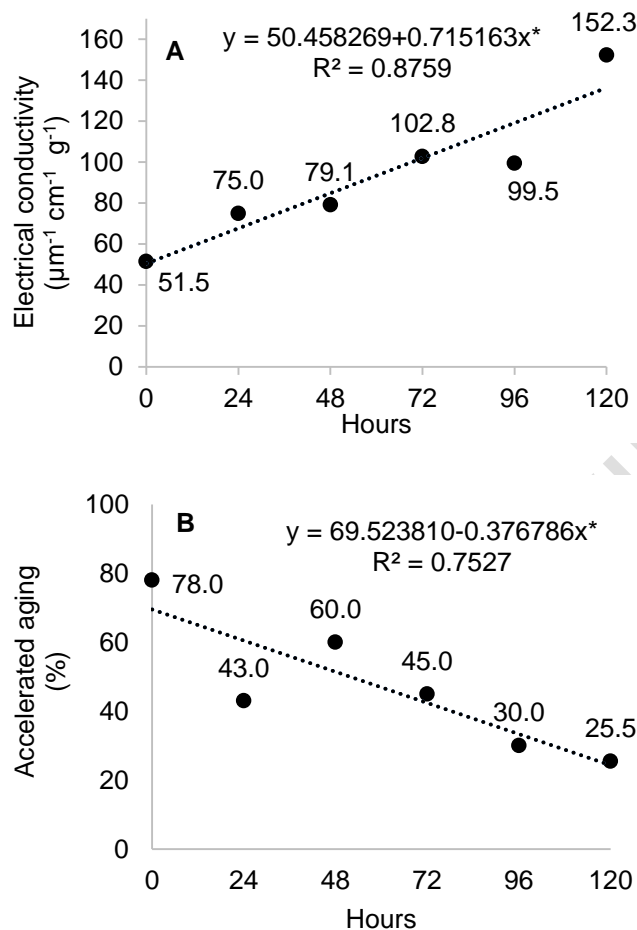


**Fig. 1.** Germination percentage (A), first germination count (B) and viability (C) of *Brachiaria brizantha* cv. *Piatã* seeds according to the contact time with the NPK 05:25:15 fertilizer.

These reductions are probably due to integument rupture and embryo damage, caused by the acid pH and the salinity index of the fertilizer. Moreover, the rapid water absorption by the seed during the imbibition phase may influence germination and viability, as it causes damage to the seeds' tissues [14, 15]. Similar results were found by testing the contact time of *Brachiaria brizantha* seeds with triple superphosphate [16].

The germination percentages obtained in the standard germination test were lower than the results from the tetrazolium test (Fig.1A and 1C), which shows that part of the viable seeds did not germinate. This might have been caused by pathogen action or physiological dormancy occurring in the *Brachiaria* seeds [17, 18]. According to Normative Instruction No. 30 [5, 19], for the commercialization of *Brachiaria brizantha* seeds, germination or viability of values next to 60% are required. Therefore, this batch only reached the market standard through viability analysis.

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



**Fig. 2.** Electrical conductivity (A) and accelerated aging (B) of *Brachiaria brizantha* cv. Piatã seeds according to the contact time with the NPK 05:25:15 fertilizer.

In a study evaluating the contact of *Brachiaria brizantha* seeds with potassium chloride, the authors verified that there was a positive linear effect only on the values of electrical conductivity [20]. This increase is justified by the high salt content of the fertilizer, which caused disruption of the seed integument and release of electrolytes [20].

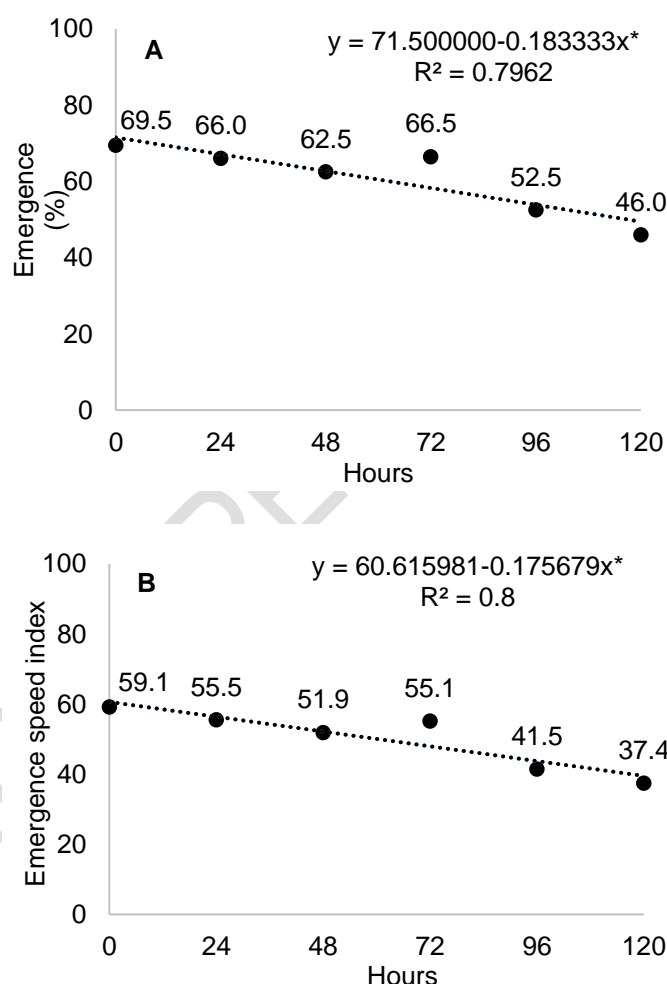
Studying seeds of *Brachiaria brizantha* cv. Marandu in contact with granulated single superphosphate, powdered single superphosphate, and granulated mono-ammonium phosphate, the authors also verified a positive linear increase in electrical conductivity values when seeds remained in touch with the single superphosphate, either granulated or powdered [21].

As the contact time of the Piatã grass seeds with the fertilizer increased, there was a 65.0% reduction in the germination of the seeds subjected to the accelerated aging test when comparing the contact time of 120 hours with no contact (Fig. 2B), pointing to a decline in the seed vigor by increasing the contact time of the seeds with the fertilizer. However, for the seeds that had no contact with the fertilizer (time zero) there was a higher germination percentage in the accelerated aging test (69.5%) than in the standard germination test

187 (57.7%). That was probably caused by the break of physiological dormancy or by pathogen  
188 level reduction due to the high temperatures in the accelerated aging test.

189 When analyzing seeds of *Brachiaria brizantha* cv. MG-5 Vitória, the authors concluded that  
190 accelerated aging is a method capable of overcoming physiological seed dormancy [22].

191 With regard to the emergence of the Piatã grass seedlings, there was a 30.8% decrease  
192 when comparing a 120 hours contact time with no contact time (Fig. 3A). When comparing  
193 these results with those obtained in the standard germination test (Fig. 1A), a higher  
194 emergence percentage can be observed, suggesting that the seeds in the sand, in the  
195 emergence test, were better able to express their productive potential, as the values were  
196 closer to those in the viability test (Fig. 1C).



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198

199 **Fig. 3.** Emergence percentage (A) and emergency speed index (B) of *Brachiaria brizantha*  
200 cv. Piatã seeds according to the contact time with the NPK 05:25:15 fertilizer.

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202 This effect may occur due to the increase in the seed contact surface with the sand or by  
203 different conditions for the development of pathogens present in the seed surface. In a study  
204 evaluating *Brachiaria brizantha* seeds in contact with potassium chloride, the authors



205 obtained similar results to those found in this work, in which the emergence percentage was  
206 higher than the germination percentage [20].

207 The values found for the emergence speed index (ESI) decreased when the seed contact  
208 time with the fertilizer was increased, following a negative linear pattern, with a 34.8%  
209 decrease when comparing a 120 hours contact time with the control time (Figure 3B). Similar  
210 results can be observed in studies testing *Brachiaria brizantha* seeds contact time with the  
211 04-14-08 NPK formulation [23].

212 Considering that the necessary commercial requirements for *Brachiaria brizantha* seeds are  
213 only acceptable with minimum germination or viability rates of 60% [19, 24], the standard  
214 was maintained with the viability test up to 85 hours after mixing the seeds with the 05:25:15  
215 NPK fertilizer.

216 With regard to germination, a result below the minimum standard required for  
217 commercialization (60 % germination) [5] was verified for all treatments.

218 However, both the viability [19] and the emergence test [6] can be adopted as commercial  
219 standards, with the minimum level for commercialization being 60% of viability or  
220 emergence. Based on the results of the viability and emergence tests, commercialization  
221 levels are maintained (60% viability or emergence) by performing the fertilizer/seeds mixture  
222 up to 63 hours before sowing.

223 The minimum standard (60% germination, viability or emergence) required for the  
224 commercialization of forage seeds should be maintained in order to ensure the ideal forage  
225 population in the crop establishment. Below these levels (under 60% germination, viability or  
226 emergence) there will be no adequate stand, and degradation of pasture and soil may occur.

227 Electrical conductivity and accelerated aging variables are indicative parameters of seed  
228 vigor. With 63 hours of seed and fertilizer mixing, there will be a reduction of approximately  
229 23% in the initial vigor of the seeds (Fig. 2). This vigor reduction will neither compromise the  
230 crop establishment nor cause degradation of soil.

231 Therefore, it may be stated that for large cropping areas, the Piatã grass seeds can be  
232 mixed with the 05:25:15 NPK formulation up to 63 hours before sowing [19, 24].

233 In a work evaluating *Brachiaria brizantha* seeds in contact with phosphate fertilizers,  
234 potassium chloride and powdered formulations, the authors observed a negative effect on  
235 seeds with a 96-hour contact time for all studied fertilizers [4].

#### 236 237 4. CONCLUSION 238

239 The minimum standard of 60% germination, viability or emergence required for the  
240 commercialization of forage seeds should be maintained to ensure the ideal forage  
241 population in the crop establishment.

242 Germination rates did not reach the minimum standard required for commercialization in all  
243 treatments.

244 Based on emergence and viability tests, commercialization standards are maintained by  
245 performing the fertilizer/seeds mixture up to 63 hours before sowing.



246 After 63 hours of seeds and fertilizer mixing, a reduction in the initial vigor of the seeds tends  
247 to occur, but there is no reduction or unevenness in the crop establishment or degradation of  
248 soil.

249  
250 Piatã grass seeds can be mixed with the fertilizer up to 63 hours before sowing.

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## 253 **COMPETING INTERESTS**

254

255 We declare that no competing interests exist.

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