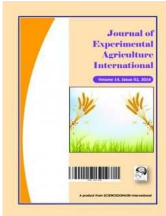

Journal of Experimental Agriculture International

18(4): 1-8, 2017; Article no.JEAI.36768
Previously known as American Journal of Experimental Agriculture
ISSN: 2231-0606

Germination Performance of Campo Grande (*Stylosanthes capitata / macrocephala*) Stylers Seeds Coated with Different Layers of Inert Material

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Authors' contributions

This work was carried out in collaboration with all authors. Author FWAS conducted all the experiments and conducted the written part with the supervision of the author HDV. Authors DFB, MQM and AJA assisted in all stages of greenhouse, laboratory and complemented bibliographical research. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JEAI/2017/36768
Editor(s):
(1) Slawomir Borek, Department of Plant Physiology, Adam Mickiewicz University, Poland.
Reviewers:
(1) Salisu Muhammad Tahir, Kaduna State University, Nigeria.
(2) José L. Pantoja, Ecuador.
Complete Peer review History: http://www.sciencedomain.org/review-history/21740

Original Research Article

Received 14th September 2017
Accepted 30th October 2017
Published 4th November 2017

ABSTRACT

The coating is based on the deposition of inert material in successive layers that allow the modification of the physical characteristics of the seeds. However, this procedure can compromise the physiological characteristics. In this sense, *Stylosanthes capitata / macrocephala* cv. Campo Grande seeds were coated with sand of less than 0.25 mm with different layers with the following treatments: TR1- Seeds not covered and scarified (SNCS); TR2 - 6 layers (150 g); TR3 - 8 layers (200 g); TR4 - 10 layers (250 g); TR5 - 12 layers (300 g); TR6 - 14 layers (350 g) and TR7 - Seeds not covered and not scarred (SNC and NS), to study the effect of the coating with different layers on the physical and physiological characteristics of these seeds. Polyvinyl glue (PVA) was used as a cementitious material. The coating was done in a bench drawer. The tests were arranged in a germitest* roll paper. The experimental design was a completely randomized, in the laboratory and randomized blocks in a greenhouse with 6 treatments containing four repetitions of 50 seeds in four

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blocks. The 12-layer sand coating benefited the physical characteristics and did not compromise the physiological characteristics of the seeds and the development of the Estilosantes Campo Grande seedlings.

Keywords: Bench drawer; polyvinyl; sand; seedlings.

1. INTRODUCTION

The growing demand for species that can consortium with Poaceae has been increasing. Currently, the genus that has been emerging to be used in a consortium with the purpose of incorporating higher levels of dry matter and protein source in pastures is the Stylosanthes. This genus belonging to the Fabaceae family there are several species that can be used for this purpose, with emphasis on two *Stylosanthes capitata and S. macrocephala* species. The highlight for these species occurs according to productive aspects, even in nutrient-deficient soils, high resistance to anthracnose, good palatability and excellent N fixation [1]. In the year 2000 the Embrapa Cutting Cattle released a cultivar of the physical mixture of these two species called Estilosantes Campo Grande (*Stylosanthes capitata / macrocephala*) [2].

However, the seeds of this cultivar are relatively small and of a very varied coloration, which makes it difficult to identify the seeds in the groove or pit during sowing, which potentiates the waste and causes damage to the producer. This waste could be minimized if the seeds had larger size and colouring of easier identification.

In this context, the coating appears as the technique that can be used to alter the physical characteristics of these seeds. In addition, it is possible to add macro and micronutrients, insecticides, fungicides, growth regulators among other elements that can benefit the seeds [3].

However, the use of the coating technique may adversely affect the physiological characteristics of the seeds depending on the type of material and amounts that are used to form the coating layers. Studies carried out by [4] on lettuce seeds report that there is influence of the materials used in the coating on the speed and on the final germination rates.

Previous studies [5] verified that seeds covered by Xaraés and *Urochloa ruziziensis* cv. Kennedy have a slow soaking, resulting in lower percentages of germination. Other studies point to the negative influence of the coating [6,7,8].

However, even if, in these studies, the seeds have suffered a negative influence of the coating, more detailed studies to determine suitable methodologies for the material can minimize the negative effect of the coating on the physiological characteristics (Germination test (G), first germination count (FGC), dead seeds (DS), soaked seeds (SS), hard seeds (HS) and abnormal seedlings (AS).

Therefore, this work aims to evaluate the physical and physiological characteristics of Campos Grande style seeds, covered with sand with different amounts of coating layers.

2. MATERIALS AND METHODS

The experiment was carried out at the State University of Northern Fluminense (UENF) in Campos dos Goytacazes - RJ, with Commercial seeds of cv. Campo Grande, which were submitted to manual scarification between two sheets of sandpaper number 100.

Sand of less than 0.25 mm in diameter was used as the coating material. To carry out the coating step, it was necessary to pass the sand in a 60 mesh sieve (0.25 mm aperture), thus separating the finer granulometry material (0.25 mm) to facilitate recoating and standardize the particle size. A solution of glue based on polyvinyl acetate (PVA) and water heated to 70℃ was used as cement. The water was first heated and then the glue was added in the ratio of 1/1 (v/v) [6].

The proportion of filler and seed material used was: TR2 - 1.5: 1; TR3 - 2: 1; TR4 - 2.5: 1; TR5 - 3: 1 and TR6 - 3.5: 1. The treatments were composed satisfying this criterion of proportionality, which resulted in the following treatments: TR1- Seeds not covered and scarified (SNCS); TR2 - 6 layers (150 g); TR3 - 8 layers (200 g); TR4 - 10 layers (250 g); TR5 - 12 layers (300 g); TR6 - 14 layers (350 g) and TR7 - Seeds not covered and not scarred (SNC and NS).

For the coating procedure, the filler materials were divided into layers of 25 g. To facilitate the coating procedure, the material of each layer (25 g) was divided into 12.5 g portions.

The seed coating process was carried out in a table top drawer, model N10 Newpack®. The equipment was set to the speed of 90 rpm, the pressure of the compressed air system at 4 bar, the drying system at 50℃. The equipment settings and recoating methodologies were adapted from [9]. An amount of 100 g of seeds per replicate was deposited into the hopper vessel, then a 12.5 g portion of coating material was placed and, finally, the compressed air system of the gun (spray), with cementitious material, was activated towards the seed mass for 3 seconds. Subsequently, a further 12.5 g of coating material was added and the material was laid for further 3 seconds and then the drying system was activated for 90 seconds. This procedure corresponded to a coating layer and was repeated until the amount of each treatment was totalized.

The evaluation occurred in the laboratory and was performed according to [10] the water content (WC) and the weight of one thousand seeds (WOT). Maximum diameter (MAD), minimum diameter (MID), Circularity (CIR) and contour irregularity (CI) were performed using the equipment *GroundEye®* system of analysis by *software*.

Germination test (G): It was carried out in a laboratory, in a completely randomized design with four replicates of 50 seeds of each treatment on substrate paper roll containing two sheets of germitest* paper, previously moistened with deionized water equivalent to 2.5 times its weight. After assembling the tests, the paper rolls were arranged in a germinator at 35 - 20℃ with photoperiod of 8 hours of light and 16 hours of darkness, for a period of 10 days [10]. Daily counts were made to calculate the germination speed index (GSI) according to the formula proposed by [11]. On the fourth day, the first germination count (FGC) and the last germination count were done to determine germination percentage (G), dead seeds (DS), soaked seeds (SS), hard seeds (HS) and abnormal seedlings (AS).

Emergency test (E): It was conducted in a greenhouse in four randomized blocks, with 50 seeds in each treatment, which were arranged in plastic trays with a capacity of 2.5 L, containing as substrate sifted and washed sand. The test lasted 30 days and the evaluations were daily to determine the emergency speed index (ESI) according to the formula proposed by [11]. At the end of the test, ten representative plants of the plots were separated for the root length (LR) and aerial part (LAP) evaluations to be carried out, using a millimeter ruler. After the biometry of the aerial parts and the roots, both were packed in bags that were previously identified and weighed in a precision scale, for the determination of the fresh mass of the aerial part (FMAP) and the root (FMR). The sacks were then kept in a forced air circulation oven at 65℃ for 72 hours to determine dry mass of the aerial part (DMAP) and root dry mass (DMR) [12].

The data for GSI, WC, AP and DS were transformed to $\sqrt{x}$, 1 / x, arcsin $(x / 100)^{1/2}$ and $\sqrt{x}$ respectively, as they did not meet the requirements of homogeneity of variance and normality by the Bartllett and Shapiro-Wilk tests, respectively. After the transformation, the requirements were met, and then the analysis of variance of the data was performed. However, the presented values refer to the original data.

The other variables followed the model of homogeneity and normality, being unnecessary transformations. The analysis of variance and averages compared by the Tukey test at the 5% probability level were performed using the ASSISTAT 7.7 beta software [13].

3. RESULTS AND DISCUSSION

When analyzing the weight data of one thousand seeds (WOT) (Table 1), it was verified that the lowest values were for treatments 1 (scarified SNC) and 7 (non-scarified SNC) in relation to the other treatments. This lower value of (WOT) for these treatments was expected, because as the formation of coating layers occurs, there is an increase in weight as a function of the amount of material deposited on the seeds. The increase in (WOT), according to [6] provides improvements to the covered seeds. This is mainly due to the ease of sowing and application of products via seeds.

By analyzing the WOT data in a descriptive way, it is possible to observe that there is a gradual increase of the treatment number 2 (6 layers of sand) to the treatment number 5 (12 layers of sand), with a decrease in weight of treatment 6 (14 layers of sand). This increase in (WOT) is due to the overlaps of the layers with the coating material, as mentioned previously. For treatment number 6 (14 layers) it could be expected that the increase would occur due to the greater amount of coating material compared to treatment 5 (12 layers). However, the extra 50 g of material corresponding to the 13th and 14th layers were not adhered to the seeds, and also impaired the 12th layer that was formed, causing peeling of the coating material which resulted in a decrease of 14% in WOT.

A study conducted by [14] found lower WOT in Brachiaria brizantha covered with sand. In this study, 400 g of coating material applied in 16 layers were used. Thus, it is shown that there is a direct relationship of WOT gain to some extent and from there the increase in the amounts of layers to the sand can negatively affect the recoating. The data indicate the saturation of sand coating material (0.25 mm) when the coating reaches the amount of 12 layers.

Analyzing the water content data (WC) (Table 1), it was verified that the treatments 1 (scarified SNCS) and 7 (un Scarified SNCNS) did not differ from each other (P = .05). However, they did differ from the other treatments. The highest value of AT in uncoated seeds was already expected, due to the fact that, as the seed coat formation occurs, there is a weight gain without gain of water, even with the sprinkling of cement material that has water in its composition. According to [15] seeds that have layers of coating with high water contents must be immediately put to dry due to the film of water retained in the pores, which can impair the gas exchanges. However, such drying should be done in a way that does not cause secondary dormancy in the seeds. In this work, it can be verified that the coating reduced the water content of the seeds, which let to a gradual decrease in the WC of the coated seeds, showing that there is a relation between the number of layers, water content and weight of one thousand seeds.

For the (MAD) and (MID) variables (Table 1), it was verified that all treatments of covered seeds differed (P = .05) from the control treatments (1 and 7). This increase is also a result of the amount of material used to compose the treatments, and this increase provides greater uniformity in seed size, since the difference in MAD and MID values is relatively small for all treatments, thus showing greater coating uniformity. According to [9], the sand gives the coating a greater increase in MAD and MID, whose increase in the values is a result of the size of the particles of sand material used in this work, which is 0.25 mm.

The circularity data (CIR), still in (Table 1), the data indicated that as the seeds were covered, these were acquired in circular format. The treatments that obtained averages with higher values were again treatments 5 and 6 (and did not differ among themselves P = .05), showing that the coating with 12 and 14 layers, besides increasing the size of the seeds, still makes the seeds more circular. These modifications in the physical characteristics of the seeds are of fundamental importance for a standardized sowing, since the distribution and identification of the seeds in the groove or the pit is facilitated in function of the size, format and color of the seeds, besides exempting the thinning for some crops. Due to that, the coating gives the seed a rounded shape, increasing its size and thus facilitating its distribution, be it manual or mechanical [4].

Previous studies [16] observed that to sow lettuce seeds covered in a tray of 128 cells, 2:12 minutes were necessary on average. However, when the seeds were bare, it took an average of 3:42 minutes, in addition to some cells with more of a seed that would later require thinning, which leads to increased costs.

The contour irregularity (CI) (Table 1), it was shown that the uncovered seed format (TR1 and TR7) had the lowest mean values and did not differ (P = .05) from each other. This behaviour indicates that, for these seeds, the irregularity is lower. This performance was expected, because the uncoated seeds present a more uniform surface due to the type of integuments that the species presents. Thus, when the coating over the seed coat occurs, there is an irregularity due to the accumulation of material, especially if it has a large particle size such as sand (0.25 mm).

This CI can be better understood when we analyze the means with the highest values (TR2 and TR6), which did not differ (P = .05) from each other. Since treatment 2 may have presented this greater irregularity mainly due to the smaller amount of material used to compose the 6 layers (150 g), and for treatment 6 the material that would form the 13th and 14th layers may have triggered detachment of the material, resulting in the unevenness of the coating and, consequently, higher CI.

Table 1. Weight of a thousand seeds (WOT), Water Content (WC) Maximum Diameter (MAD), Minimum Diameter (MID), Circularity - CIR, Contour irregularity – IC of seeds of cv. Campo Grande covered with sand (0.25 mm) with different layers

	WOT	WT	MAD	MID	CIR	CI
	g	%		mm		
TR1	2.6	8.3 A	2.4 D	1.4 D	6.0 C	0.25 C
TR2	5.2	6.6 B	2.5 C	1.7 C	6.8 B	0.41 A
TR3	5.4	6.1 BC	2.7 C	1.7 C	6.7 B	0.37 B
TR4	6.1	4.9 CD	2.9 B	1.9 B	6.8 B	0.33 B
TR5	8.5	3.6 D	3.2 A	2.2 A	7.2 A	0.34 B
TR6	7.3	4.7 CD	3.2 A	2.2 A	7.1 A	0.44 A
TR7	2.7	9.1 A	2.4 D	1.4 D	6.0 C	0.24 C
CD (*p=.05*)	**-**	**1.67**	**0.19**	**0.85**	**2.81**	**0.38**
CV (%)	**-**	**11.77**	**1.68**	**1.93**	**1.83**	**4.82**

**SCD (p =.05) means critical difference among treatments at 5 per cent level of significance TR1 - Seeds not covered and scarified (SNCS); TR2 - Sand 6 layers (150 g); TR3 - Sand 8 layers (200 g); TR4 - Sand 10 layers (250 g); TR5 - Sand 12 layers (300 g); TR6 - Sand 14 layers (350 g); TR7 - Non-scarified seeds (SNCNS)*

The first germination test (GPC) (Table 2) showed that there was a significant difference (*P = .05*) between treatments 3, 5 and 7, and the mean of treatment number 5 was the one with the highest value. However, it did not differ significantly (*P = .05*) from treatment 1 of uncovered and scarified (SNC and scarified) seeds, as well as treatments 2 (12 layers), 4 (10 layers) and 6 (14 layers). This statistical similarity (*P = .05*) with treatment 1 indicates that the coating, even with different numbers of layers, did not cause a deleterious effect on the vigor of the seeds, as well as promoted a numerical increase, improving this characteristic, except for TR3.

According to [17] the first germination count test, by the ease of execution, can be used to obtain preliminary seed germination information, allowing to evaluate the vigor of a seed lot. Several authors, when evaluating the effect of the seed coating, observed that there were no differences between the seeds covered with the control, showing that the well-conducted coating does not interfere in the physiological quality of the seeds [4,9,14,18,19,20].

When analyzing the mean of treatment number 7 (SNC and non-scarified), the importance of scarification of the seeds before recoating was confirmed, due to the delay and reduction of germination caused by the tegumentary dormancy peculiar to the species.

In Table 2, it is verified that the majority of the treatments with coating had a negative effect in the variable. Only treatment number 5, composed by 12 layers, did not differ from treatment 1 (control). Possibly, even if the seeds were scarified, the coating caused less permeability in the seeds. This lower permeability of the coating could certainly be related to the cementitious material, and not to the coating material, since treatment 5, which presented the highest WOT, did not differ from treatment 1 (control) in the GSI variable. It is probable that the proportion of filler material and cement in 12-layer seeds has formed a more uniform layer with a greater amount of pores, thus allowing more gas exchange and diffusion of water between the external medium and the seeds, unlike the other treatments that impaired the GSI.

According to [16] the PVA glue (polyvinyl acetate) has good particle aggregation ability of the material used to cover the seeds. However, it can form a thick layer that can hinder the diffusion of water and the gas exchanges between the seeds and the environment, and it is important to minimize the use of PVA glue. This is due to the dehydration characteristic of the cementitious material, which is potentiated by the drying of the seeds, making the cementing material more compact and less permeable [4].

Even with higher solidity and lower permeability, the seedlings that did not germinate were able to absorb water as observed in Table 2, in the seed variable (SS). It is important to emphasize that, regardless of the physiological quality of the seeds, this process occurs due to the negative hydric potential of the seeds. However, a too slow or too rapid diffusion of water into the seeds can promote physiological insults during imbibition due to the state in which the membranes meet [21]. Thus, the impermeability, even when it does not significantly affect the FGC, G and SS variables, can result in significant values of DS. Indeed, it is indicative that the physiological tests for coated seeds should be conducted at longer time intervals than RAS [10] recommends for uncoated seeds.

Although the TR3 and TR4 coatings increased the percentages of dead seeds, this effect was not observed in the percentage of abnormal seedlings (AS), nor of hard seeds (HS).

The germination data (G) (Table 2) point to very promising results, since different coating treatments presents values significantly equal to the uncoated seeds, highlighting treatment number 5 with a numerically higher average than the other treatments. It is important to note that treatments 2, 4 and 6 presented statistically different means of treatment 1 (control) for GSI, but did not differ for germination (P = .05). This behaviour indicates that even if the coating had contributed to a lower value in the GSI of these treatments, the seeds were able to overcome the physical barrier imposed by the coating over time and also to establish themselves as normal seedlings.

The main recurring problem in the use of coated seeds is the delay caused by the physical barrier [22]. Thus the importance of studying the germination process as a function of the coating material, since the type of material can significantly compromise the final germination.

The results of greenhouse experiments are shown in Table 3. It is observed that the results of emergency percentage (E) indicate that the recovered seeds did not differ statistically from the control treatment. Only the uncoated seeds without scarification presented an average value lower than the control treatment, indicating the importance of the mechanical scarification used to create cracks in the integument of the seeds, cracks which facilitate the diffusion of water from the external medium to the seeds. According to [23] integument dormancy causes impermeability of the integument to water and gas exchange, resulting in integument dormancy.

Coating treatments were effective for sowing in the field, as they did not significantly interfere *(P = .05)* in emergence compared to TR1. This effect can be the result of the arrangement that the sand particles formed, providing larger porous spaces, that allowed the diffusion of water and gases. According to [24], materials that have larger particle size form aggregates with larger pores, different from materials that have smaller particles that form aggregates with smaller pores. Thus, the treatments proposed in this study, using as sand coating material in the granulometry less than 0.25 mm, provided statistically the same values as those of the uncovered and scarified seeds.

Also in Table 3, it can be observed that the values of the emergency speed index (ESI) did not differ significantly *(P = .05)*. These values point once more to the effectiveness of the coating to the maintenance of the physiological characteristics of the seeds. Generally, the coating results in lower ESI values, as described by several authors [4,6,20]. However, this effect was not observed in this study, since the ESI's of

Table 2. Index of germination speed (GSI), First germination count (FGC) Percentage of germination (G); Soaked seeds (SS); Dead seeds (DS); Abnormal seedlings (AS); Hard seeds (HS): of seeds of styles cv. Campo Grande covered with sand (0.25 mm) with different layers

	GSI	FGC	G	SS	DS	AS	HS
		%					
TR1	25.6 A	61.5 AB	73.5 A	15.0 B	0 C	6.0 A	5.5 A
TR2	17.9 CD	66.5 AB	69.0 AB	20.5 AB	4.5 BC	4.0 A	2.0 A
TR3	14.8 CD	52.0 B	58.0 B	28.5 A	5.5 B	6.5 A	1.5 A
TR4	19.5 BC	66.5 AB	68.5 AB	10.2 B	12.7 A	5.5 A	3.0 A
TR5	22.7 AB	73.5 A	76.5 A	16.7 AB	0.25 BC	3.5 A	3.0 A
TR6	13.8 D	68.5 AB	70.0 AB	21.0 AB	0.5 BC	5.5 A	3.0 A
TR7	8.4 E	33.0 C	35.0 B	52.0 A	2.5 BC	6.5 A	4.0 A
CD (*p=.05*)	**4.84**	**18.30**	**14.34**	**13.47**	**5.29**	**7.35**	**4,07**
CV (%)	**13.22**	**12.02**	**9.69**	**25**	**22.03**	**32.72**	**26.4**

**SCD (p =.05) means critical difference among treatments at 5 per cent level of significance TR1 - Seeds not covered and scarified (SNCS); TR2 - Sand 6 layers (150 g); TR3 - Sand 8 layers (200 g); TR4 - Sand 10 layers (250 g); TR5 - Sand 12 layers (300 g); TR6 - Sand 14 layers (350 g); TR7 - Non-scarified seeds (SNCNS)*

Table 3. Emergency (E); Index of speed of emergency (ESI); Length of aerial part (LPA); Root length (LR); Fresh aerial mass (FMPA); Aerial dry mass (DMPA); Fresh root pasta (FMR); Root dry mass (DMR): from seeds of cv. Campo Grande covered with sand (0.25 mm) with different layers

	E	**ESI**	**LPA**	**LR**	**FMPA**	**DMP**	**FMR**	**DMR**
	%		**cm**		**mg/pl**			
TR1	58.5 A	6,61 A	0,83 A	8,6 A	478,8 A	82,3 A	371,8 A	39,3 A
TR2	54.5AB	6,31 A	0,69 A	8,0 A	435,3 A	69,8 A	355,0 A	34,0 A
TR3	51.0AB	6,02 A	0,88 A	8,8 A	470,5 A	78,5 A	426,8 A	40,8 A
TR4	54.5AB	6,56 A	0,68 A	5,7 A	419,3 A	65,8 A	303,3 A	29,0 A
TR5	50.0AB	5,83 A	0,82 A	9,0 A	489,5 A	82,8 A	358,3 A	40,3 A
TR6	4.5AB	6,41 A	0,68 A	8,2 A	409,3 A	61,3 A	344,3 A	31,0 A
TR7	28.5 B	3,21 A	0,80 A	8,2 A	440,8 A	106,5 A	365,8 A	40,8 A
CD (*p=.05*)	**29.37**	**3.59**	**0.36**	**6.72**	**14.21**	**35.01**	**28.78**	**2.11**
CV (%)	**21,85**	**33,84**	**20,42**	**34,16**	**13,91**	**41,44**	**30,75**	**23,17**

**SCD (p =.05) means critical difference among treatments at 5 per cent level of significance TR1 - Seeds not covered and scarified (SNCS); TR2 - Sand 6 layers (150 g); TR3 - Sand 8 layers (200 g); TR4 - Sand 10 layers (250 g); TR5 - Sand 12 layers (300 g); TR6 - Sand 14 layers (350 g); TR7 - Non-scarified seeds (SNCNS)*

the coated treatments did not differ from the control treatment (TR1). This statistical similarity (*P = .05*) between the greenhouse test treatments may have been a consequence of higher temperatures and frequent irrigation, which promoted greater solubilization of the coating, accelerating the emergency in comparison with the ESI performed in the laboratory.

For the aerial part (LAP), root length (LR), fresh mass of the aerial part (FMAP), dry mass of the aerial part (DMAP), root fresh mass (FMR) and root dry mass (DMR) variables, there was no statistical difference *(P = .05)*, demonstrating that the coating of the styling seeds did not affect the development of the plants during the evaluated period. These results corroborate to consolidate the beneficial effect of the coating with sand with particles smaller than 0.25 mm.

4. CONCLUSION

The coating with sand benefited the physical characteristics and did not compromise the physiological characteristics of the Stylosanthes seeds Campo Grande. The best coating of seeds was with 300 g of sand, corresponding to 12 layers of coating. The covering with sand did not compromise the development of the seedlings of Stylosanthes Campo Grande.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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