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Cientific Paper

Physiological potential of legumes seeds submitted to stay in mineral mixture

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Abstract

Objective of this study was to evaluate the potential physiologic potential of seeds of four tropical forage legumes submitted to the permanence in mineral mixture for bovine, for the germination test, for the accelerated aging test and for the electric conductivity test, with the intention of dispersing them in the pasture by the bovine feces. A completely randomized design at factorial scheme 4x7 (four species, seven treatments) with four repetitions was used. The seeds stayed in the mineral mixture for: 0, 12, 24, 36, 48, 72 and 96 hours. The saline stress, up to 24 hours, did not represent risk for the germination of the seeds. Like this, the introduction of seeds in mineral mixture for bovines for dispersion of legumes in the pasture can used.

Key words: Mineral supplement; germination; humidity rate and vigor.

Resumo

Potencial fisiológico de sementes de leguminosas submetidas à permanência em mistura mineral

Objetivou-se com estudo avaliar o potencial fisiológico de sementes de quatro leguminosas forrageiras tropicais submetidas à permanência em mistura mineral para bovinos, pelo teste de germinação, pelo teste de envelhecimento acelerado e pelo teste de condutividade elétrica, com o intuito de dispersá-las na pastagem pelas fezes bovinas. O delineamento experimental foi inteiramente casualizado em esquema fatorial 4x7 (quatro espécies, sete tratamentos), com quatro repetições. As sementes permaneceram na mistura mineral por: 0, 12, 24, 36, 48, 72 e 96 horas. O estresse salino, até 24 horas, não representou risco para a germinação das sementes. Assim, a introdução de sementes em mistura mineral para bovinos para a dispersão de leguminosas na pastagem pode ser utilizada.

Palavras chave: Suplemento mineral; germinação; teor de umidade e vigor.

Resumen

Potencial fisiológico de semillas de leguminosas sometidas a la permanencia en mezcla mineral

Se objetivó io evaluar el potencial fisiológico de semillas de cuatro leguminosas forrajeras tropicales sometidas a la permanencia en mezcla mineral para bovinos, por el test de germinación, por la prueba de

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envejecimiento acelerado y por la prueba de conductividad eléctrica, con el propósito de dispersarlas en el pastoreo por las heces bovinas. El delineamiento experimental fue completamente casualizado en esquema factorial 4x7 (cuatro especies, siete tratamientos), con cuatro repeticiones. Las semillas permanecieron en la mezcla mineral por: 0, 12, 24, 36, 48, 72 y 96 horas. El estrés salino, hasta 24 horas, no representó riesgo para la germinación de las semillas. Así, la introducción de semillas en mezcla mineral para bovinos para la dispersión de leguminosas en el pastoreo puede ser utilizada.

Palabras clave: Suplemento mineral; germinación; contenido de humedad y vigor.

Introduction

Several researchers have done the study evaluating forage legumes in order to take advantage of them in pastures; however, one of the biggest problems encountered is the establishment phase. According to Valentine and Carneiro (1998) and Tonin (2004), the cheapest way to introduce legumes into pastures is to use cows as "planters", ie the producer introduces seeds in the diet of cows or mineral salt to cattle to occurring the breaking of dormancy, and the animals themselves spread. Although, several studies have conducted to assess various forms of introduction of legumes in pasture (VALENTINE et al., 2002; RIBEIRO et al, 2007). There are few studies aimed at the development of seed dispersal technique through introducing seed concentrate or mineral mix for cattle, goats, sheep and / or horses (DEMINICIS et al. 2007; SILVA, 2008, ALMEIDA et al, 2015).

On the mechanisms of adaptation to salinity, many studies have conducted regarding the physiology of plant resistance to salinity (WANG and NIL 2000). In addition, the high salinity biological responses in plants have been more recently discussed (MUNNS, 2005). The evaluation of the physiological potential of the seeds is a key component of determining the quality of seeds, as is reference on the performance of plants (TORRES and NEGREIROS, 2008).

For this, various procedures have used, including the accelerated aging test and electrical conductivity, using the solution imbibition of seeds. The accelerated aging test evaluates the reaction of seeds when exposed to high temperature and humidity. The conductivity test based on the principle that with the deterioration process is the leaching of the cellular constituents of seeds soaked in water due to loss of integrity of the cellular systems. Thus, low conductivity means high quality seed and high conductivity, i.e., greater output seed leachate suggests the lowest force thereof, due to increased electrolyte (VIEIRA and KRZYZANOWSKI, 1999). As a result, this study aimed to evaluate the physiological potential of seeds of four tropical forage legumes submitted to the mixture continuously mineral for cattle, in order to provide seed bovine mixed mineral salt in order to use as intake vehicle to disperse them in the pasture cattle dung.

Material and Methods

The experiment conducted in the Plant Science Laboratory UENF in Campos dos Goytacazes County in Rio de Janeiro State, Brazil, the species used: Clitorea ternatea, Stylosanthes spp. cv. Campo Grande, Neonotonia wightii and Macrotyloma axillare.

The experimental design completely randomized in a 4x7 factorial used (four pulses, 7 periods of stay), with four replications. The treatments were: A) stay in mineral mixture for cattle by: A) 12; B) 24; C) 36; D) 48; E) 72; E) 96 and F) Zero hours. The composition of mineral mixture "full" for beef cattle was as follows, per kg of product: Ca = 172.93 g; P = 41.8 g, Na = 157.09 g; Mg = 7.14 g; S = 26.39 g; Fe = 1598.8 mg; F = 418 mg; Co = 80 mg; Cu = 1250 mg; I = 97.6 mg; If = 37.5 mg; Zn = 3800 mg; Mn = 764.4 mg and P Solubility in BC. Citric 2% = 90%. Since the sodium, content of the used mineral mixture (15%) similar to that found in white salt content for cattle (16%).

For the preparation of treatments used 200 grams of mineral mixture and 20 grams of seeds for each period of stay, by repetition. After treatment, 50 seeds per replication washed with distilled water (four washes with 50 ml), dried with paper towel and scarified with sandpaper nº 100, to observed slots in husk of the seeds to break dormancy.

After scarification, the germination test to determine the effects of salt stress on the germination percentage (PG%) and germination speed index (GSI) was performed when the seeds were placed in germination chamber type BOD at 25 ° C with 12 hours of light for 10 days. daily counts of germinated seeds were carried out. The GSI calculated as Maguire (1962). To determine the effects of salt stress on the moisture content of the seeds (U%), after treatment, the seeds were weighed and taken in aluminum containers for dehydration oven (105ºC). For the determination of moisture content, the formula was used: U% = ((Pstart - Pfinal) x 100) / Pstart.

In GSI separated 5g seeds of each species per treatment (residence periods in mineral mix), for repetition, which washed with distilled water (4 rinses with 50 ml), dried towel and placed on paper "gerbox" containing 40 ml of distilled water (MARCOS FILHO et al., 1987), maintained at 41 ° C for periods of 48 hours (GARCIA and MENEZES,1999). After this aging period, four replications of 50 seeds per treatment as procedure described for the germination test (BRASIL, 2009).

For the electrical conductivity test, separated 50 whole seeds of each species per treatment (residence periods in mineral mix), for repetition. Which washed with distilled water (four rinses with 50 ml), dried on paper towels, weighed and placed in disposable plastic cups with a capacity of 100 ml; added 75 ml of distilled water and maintained for 24 hours in a chamber at 25ºC. After this period, we determined the conductivity of the solution in which the seeds found is immersed, the reading was done conductivity Digimed DM-31model, and the obtained value is divided by the weight of the seeds Vieira and Carvalho (1994) and the results were expressed in S / cm / g Vieira and Krzyżanowski (1999).

The results of germination expressed in percentage and submitted to analysis of variance, using the Tukey test at 5% significance for the comparison of means. The results processed for analysis of variance in arcosen√x / 100, except the data for the germination speed index and first count and electrical conductivity.

Results and Discussion

In Table 1, observed the results for percentage of germination seeds (PG), germination speed index (GSI), water content (U%), first count of germination (1st PG), electrical conductivity (EC) and accelerated aging (AA) of butterfly pea, estilo, perennial soybean and macrotyloma submitted to different periods of salt stress in mineral mixture for cattle.

As for the percentage of germination, the values for all species decreased as they increased the period of stay in the mineral mixture, however did not show drastic reduction behavior of this percentage in the first 24 hours of salt stress, although the end of all stress period (96 hours), halve the values of the percentage of seed germination.

The reduction observed in this study was around 39.1; 45.5; 49.6 and 11.6% for butterfly pea, estilo, perennial soybean and archer, respectively. It observed that the response of species not follow a single pattern, precisely because there are variations in the relative sensitivity of the species to salt stress, as reflected in the differences observed in germination and establishment of plants of different species.

Table 1. Percentage of germination of seeds (PG), germination speed index (GSI), water content (U%), first count of germination (1st PG), electrical conductivity (EC) and accelerated aging (AA) butterfly pea, estilo, perennial soybean and archer submitted to different periods of salt stress on mineral mixture for cattle.

	PG (%)							
Species \ Hours	0	12	24	36	48	72	96	CV%
butterfly pea	87.00 ab	96.00 a	91.00 a	85.00 ab	72.00 ab	53.00 b	53.00 b	4.52
estilo	88.00 a	81.50 ab	66.00 ab	61.00 ab	61.00 ab	55.00 ab	48.00 b	9.77
perennial soybean	61.50 a	56.00 a	51.50 a	56.00 a	51.00 a	34.50 a	31.00 a	6.21
archer	64.50 a	67.50 a	65.00 a	66.00 a	67.00 a	56.00 a	57.00 a	6.26
	GSI							CV%
butterfly pea	10.55 ab	11.44 a	10.83 a	9.86 ab	8.18 ab	5.86 b	5.89 b	3.79
estilo	5.37 a	4.99 a	4.10 a	3.14 a	3.79 a	3.44 a	3.00 a	9.51
perennial soybean	5.98 a	5.40 a	4.88 a	5.44 a	4.98 a	3.27 a	3.13 a	6.17
archer	7.15 a	7.44 a	6.83 a	6.63 a	6.90 a	5.73 a	5.96 a	6.03
	U (%)							CV%
butterfly pea	22.73 a	16.66 ab	7.22 bc	6.63 bc	5.84 bc	6.06 bc	4.69 c	9.07
estilo	12.33 a	9.66 a	9.35 a	9.04 a	8.91 a	8.88 a	8.72 a	2.38
perennial soybean	25.97 a	13.14 ab	10.94 b	9.18 b	4.16 b	4.27 b	4.37 b	7.43
archer	27.76 a	20.73 ab	11.27 b	10.38 b	10.11 b	8.49 b	8.22 b	9.07

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	1st PG(%)							CV%
butterfly pea	81.00 a	77.00 ab	69.00 b	57.00 b	44.00 bc	27.00 c	29.00 c	8.79
estilo	80.00 a	73.50 a	64.00 b	47.00 c	59.00 c	55.00 c	48.00 c	9.05
perennial soybean	20.50 a	17.50 a	14.00 a	18.50 a	17.50 a	9.50 a	13.00 a	20.46
archer	42.50 a	43.50 a	34.00 a	27.00 b	31.50 b	25.50 b	29.00 b	13.44
	EC(μS/cm/g)							CV%
butterfly pea	15.97 a	17.51 a	18.04 a	23.76 a	23.98 a	23.84 a	34.62 a	7.42
estilo	234.33 b	228.20 b	244.30 b	226.80 b	223.30 b	300.13 a	334.43 a	9.07
perennial soybean	238.00 b	272.89 b	414.81 ab	417.20 ab	581.46 ab	582.65 ab	736.54 a	11.66
archer	191.96 b	375.13 a	398.37 a	394.45 a	397.95 a	402.99 a	385.63 a	3.31
	AA (%)							CV%
butterfly pea	85.77 a	94.67 a	84.47 a	71.74 ab	50.10 ab	21.98 b	15.91 b	7.91
estilo	86.78 a	79.93 ab	56.90 ab	28.25 b	37.88 b	28.48 b	23.16 b	19,61
perennial soybean	59.76 a	53.69 ab	39.95 b	36.10 b	23.80 bc	7.94 c	6.93 c	13.33
archer	62.80 a	65.56 a	55.60 a	48.47 a	45.84 a	29.25 b	35.71 b	12.76

** Means followed by the same letter in the same line do not differ by Tukey test at 5% probability.*

This behavior can explained by the fact that sharp reduction in water content coincides with the maximum germination and vigor (CARVALHO and NAKAGAWA, 2000). Another reason, according Deminicis et al. (2007), it may have been how the seeds were submitted to the mineral mixture, unlike other studies that submitted the seeds to stress in saline solutions. In contrast, the sensitivity increases in seed species with higher water content in their tissues before the stress and during the development of the plants after germination (MASS and HOFFMANN, 1977). The absorption of the seeds salt ions can determine disturbance in the osmotic balance of cells and toxicity. On the other hand, this behavior can influenced by water availability depending on the type of substrate and water potential in the same.

According to Lopes and Macedo (2008), the successful germination is dependent on the movement of water through the tissues surrounding the seed and the presence of salts affects the water potential, reducing the potential gradient between the substrate and the surface of seed, restricting water uptake by seed. Thus, Salt stress can considered as a water stress before the germination and resulting in damage to seeds and germinating them. This is because the germination characterized by radicle protrusion, which is complete only when the seed water content exceeds a critical value, which allows the activation of metabolic processes promoters of the growth of the embryonic axis (TAMBELINI and PEREZ, 1998). As the salt stress affects the quality of seeds, their response is compromised because the increased concentration of salts in the substrate determines reduction in water potential of seeds, resulting in lower capacity for water uptake by seed, which usually influences germination and seedling development (REBOUÇAS et al., 1989). For the results of the first count germination, observed that, with increasing residence time in the mineral mixture, the percentage of normal seedlings significantly reduced. This behavior is similar to that reported by Torres (2007), which studied the watermelon germination depending on the salinity found that for the results of the first count germination, increasing the osmotic potential of the germination substrate dramatically reduces the percentage of normal seedlings. The reduction observed in this study around 64.2; 40.0; 36.6 and 31.8% for butterfly pea, estilo, perennial soybean and archer, respectively. Miranda et al. (2010) evaluated the effect of increasing NaCl concentrations on imbibition and percentage of germination of 'Colombia' ecotype cape gooseberry seeds. Verified Root malformations such as lack of elongation observed in the highest NaCl concentration treatment.

Comparing the results of the first count with the final germination percentage, it found that the first count data the most affected in the wedge is estilo with increased permanence of seeds in the mineral mix. This fact already expected because the germination rate is the first parameter affected by reduced water availability (TORRES, 2007).

However, the different behavior between species may associated with the presence of hilum and micropyle largest microscopically or more sensitive in butterfly pea and estilo, perhaps the integument motif estilo not have numbness can contribute to this result. This is because the tests (seed coat), the hilum and micropyle are the main routes of entry and exit water the seeds of most legumes, although this passage of water is slow. The water content (U%) seed reduced in its content as that increased the residence time in the mineral mixture, with 0.59 average positive correlation with PG and 0.67, with the GSI. The observed reduction (between zero time and 96 hours) in this study was around 79.4; 29.2; 83.2 and 70.4% for Butterfly pea, estilo, perennial soybean and archer, respectively.

Dias et al. (2015) evaluated the effect of salinity and temperature on the germination of carrot seeds, observed that germination reduced by the temperature of 35 °C and by osmotic potentials greater than –0.8 MPa. Besides that, temperatures of 20 °C and 25 °C more favorable to the germination of seeds in the GSI salt conditions tested. However, estilo less lost water, and kept water content between 70 and 80% in the first 24 hours, with a reduction of 24.15%. In other species to reduce the water content was drastically just the first 24 hours, or wedge, the archer and perennial soybean were respectively reduced to 68.2; 57.9 and 59.4%, probably due to the higher initial U%, which facilitates the loss of water in these seed.

To the extent that increased persistence of seeds in the mineral mixture, the IVG decreased, however, the first 24 hours, the wedge increased to IVG remained stable in three other species relative to zero time. This behavior observed by Deminicis et al. (2007) working with three of the species used in this study (butterfly pea, perennial soybean and archer), submitted to sodium chloride continuously. Suggest that overcome the salt stress, and through some treatment that overcomes seed dormancy, as for example scarification tegument with sandpaper, they germinate quickly as possible, since the stress does not exceed 24 hours and conditions are favorable for germination. Queiroga et al. (2006), working with melon seeds, found that the pre-germination treatment (salt stress seed) provides benefits for germination and greater dry mass of the aerial part of the seedlings; On the other hand, salinity irrigation water reduces the leaf area and height of the seedlings of the species. Regarding the electric conductivity, it is noted that there has been an increasing trend in the amount of electrolytes released by the four seed species with the course of the soaking time; a fact reported by several authors working with soybean (MARCOS FILHO et al., 1990; DIAS and MARCOS FILHO, 1996). However, these increases are proportional to the increase in stay under salt stress, for each species over time. Thus, this response can evidenced in the observation of the average negative correlation of 0.532 with the PG, and 0.589 with the GSI. This is important, because with the difference between the seeds subjected to different periods of stress was observed, except the butterfly pea. Regarding the effect of salt ions possibly present associated with the integument of the seed, because of salt stress to which it submitted it tried to eliminate as much as washing with distilled water. Furthermore, seed size can certainly affect the evaluation of conductivity, as the wedge is three to five times larger seeds than those of other species investigated.

Guedes et al. (2011) evaluated the effects of saline stress and temperature on the germination and vigor of C. *glaziovii* seeds. Saline solutions prepared using sodium chloride (NaCl) as the solute, first count and germination speed index and seedling lengths (root and shoot) evaluated. Increasing salt concentration in the substrate reduced the germination and vigor of C. *glaziovi*i seeds, especially at temperatures of 30 °C and 35 °C. At temperatures of 25 °C and 20-30 °C, the seed germination and vigor less affected by water stress. Saline stress caused by NaCl up to the potential of 4.5 $dS.m^{-1}$ did not affect the germination of *C. glaziovii*, which has developed a high tolerance to salinity. In general, the use of seeds of different sizes affect the values of electrical conductivity. Examining the results of the accelerated aging test conducted for a period of 48 hours at 41°C, observed that the values of germination for all species decreased as it increased the residence time in the mineral mixture. Presenting. Verified positive average correlation of 0.92 with the TG and 0.94, with the IVE, and following the information obtained in the germination and first count (1st PG).

Thus, the seeds that not subjected to salt stress in mineral mixture presented themselves as the highest quality and the seeds subjected to 96 hours of stress as the lower quality. While the seeds submitted to 24 hours, similar quality from the seeds subjected to 12 hours of salt stress and seeds that not subjected to salt stress. This result assures the advantages of determining the quality of seeds by accelerated aging test. Pereira et al. (2016) evaluated the effects of saline and temperature stress on germination and vigor of *Piptadenia moniliformis* (a species known in Brazil as "catanduva") seeds, verified in terms of germination percentage and germination speed index, seedling (root and shoot) length, and total dry matter. *P. moniliformis* seeds were able to germinate under temperatures of 25 and 30°C and tolerate osmotic potentials of up to -0.6 MPa; from this point on, there is progressive decrease in the physiological quality of seedlings. However, in general, according to Marcos Filho (1999), as verified for other tests, it is difficult to identify differences between intermediate seed vigor, a fact also confirmed by Avila et al. (2006).

Conclusions

The salt stress, up to 24 hours, no risk for seed germination and can be recommended to introduce seeds on mineral mixture for cattle, for the introduction of legumes in the pasture.

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