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# Physiological quality of cowpea seeds for different periods of storage[1]

## Qualidade fisiológica de sementes de feijão-caupi em diferentes períodos de armazenamento

**Oscar José Smiderle[2], Aline das Graças Souza[3]*, José Maria Arcanjo Alves[4] and Cylles Zara dos Reis Barbosa[4]**

**ABSTRACT -** The aim of this study was to evaluate physiological quality in the seeds of four cowpea cultivars during nine months of storage. The cowpea cultivars used were BRS Mazagão, UFRR Grão Verde, Pretinho Precoce 1 and BRS Guariba, produced intercropped with cassava in the experimental area of the Centre for Agrarian Science of the Federal University of Roraima, in Boa Vista. After harvest, the seeds were dried, hand cleaned, placed in PET bottles and stored for nine months at a temperature of 23 ºC and a relative humidity of 60% at the Seed Analysis Laboratory of Embrapa Roraima. Data analysis was carried out in a completely randomised design, in a 4 x 4 factorial scheme with eight replications. The physiological quality of the seeds was evaluated at the start of storage, and after 3, 6 and 9 months, for germination, first germination count, 1000-seed weight, moisture, electrical conductivity, speed of emergence, seedling field emergence and water gain. Physiological quality in the seeds of BRS Guariba is influenced by the storage period. Irrespective of storage period, seeds of the Mazagão cultivar show superior physiological quality in relation to the other cultivars. Seeds of the BRS Guariba and Grão Verde cultivars show a reduction in physiological quality after three months of storage.

**Key words:** *Vigna unguiculata*. Water content. Test for vigour.

**RESUMO -** Objetivou-se avaliar a qualidade fisiológica de sementes de quatro cultivares de feijão-caupi durante nove meses de armazenamento. As cultivares de feijão-caupi utilizadas foram: BRS Mazagão, UFRR Grão Verde, Pretinho Precoce 1 e BRS Guariba, produzidas consorciadas com a mandioca em área experimental do Centro de Ciências Agrárias da Universidade Federal de Roraima, em Boa Vista. Após a colheita, as sementes foram secas, limpas manualmente, acondicionadas em garrafas tipo pet e armazenadas durante nove meses em temperatura de 23 ºC e umidade relativa do ar de 60%, no Laboratório de Análise de Sementes da Embrapa Roraima. A análise dos dados foi realizada no delineamento inteiramente casualizado, disposto em esquema fatorial 4 x 4 com oito repetições. A qualidade fisiológica das sementes foi avaliada, no início do armazenamento e após 3; 6 e 9 meses, quanto a germinação, primeira contagem de germinação, massa de mil sementes, umidade, condutividade elétrica, velocidade de emergência, emergência de plântulas em campo e ganho de água. A qualidade fisiológica das sementes da BRS Guariba é influenciada pelo período de armazenamento. Independente do período de armazenamento, as sementes da cultivar Mazagão apresentam qualidade fisiológica superior, em relação às demais cultivares. Sementes das cultivares BRS Guariba e Grão Verde apresentam redução na qualidade fisiológica após três meses de armazenamento.

**Palavras-chave:** *Vigna unguiculata*. Teor de agua. Teste de vigor.

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*Autor para correspondência
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[1]Pesquisa financiada pela Empresa Brasileira de Pesquisa Agropecuária (Embrapa-RR)
[2]Empresa Brasileira de Pesquisa Agropecuária - Embrapa Roraima, Boa Vista-RR, Brasil, 69.301-970, oscar.smiderle@embrapa.br
[3]Departamento de Botânica, Universidade Federal de Pelotas, Pelotas-RS, Brasil, 96.010-900, alineufla@hotmail.com
[4]Departamento de Agronomia, Universidade Federal de Roraima, Boa Vista-RR, Brasil, 69.300-000, arcanjoalves@oi.com.br, zarabarbosa@bol.com.br

## INTRODUCTION

The cowpea [*Vigna unguiculata* (L.) Walp.] is a tropical and subtropical plant which originated in Africa (FREIRE FILHO *et al*., 2011) and whose denomination or common names vary according to the region where it is established. In the northeast of Brazil it is known as *feijão-de-corda* and *feijão macassar* in the north it is known as *feijão de praia* (ALVES *et al*., 2015) and *feijão de estrada*, and in the south as *feijão miúdo* (FREIRE FILHO *et al*., 2011).

The cowpea is widely distributed throughout the world, being one of the most important sources of protein (GUILHEN *et al*., 2016; HADŽIC *et al*., 2013; PLANS *et al*., 2013) for low-income populations. Data available from the FAO (2016) on world cowpea production for 2013 show that 3.6 million tonnes were produced over 12.5 million hectares. Production takes place in 36 countries, with Nigeria, Niger (CASTELLETTI; COSTA, 2013) and Brazil being the greatest producers, accounting for 84.1% of the global area and 70.9% of global production (BRASIL, 2016).

In Brazil, the cowpea represents 35.6% of the planted area and 15% of total bean production (cowpea and the common bean - *Phaseolus vulgaris* L.). The northeast (1.2 million hectares) and north (55.8 thousand hectares) of the country are the regions that most contribute to this production (CONAB, 2016).

In the State of Roraima (RR), the cowpea is a species with productive potential, since it is a rustic plant and shows a high capacity for the biological fixation of atmospheric nitrogen, adapting to the low natural fertility of the soils in the region. In Roraima, around 1,000 ha of cowpea are cultivated each year; average productivity is of the order of 666 kg $ha^{-1}$, with most of the production being consumed by the producers themselves, and the small surplus marketed mainly in Boa Vista, RR, and in Manaus, in the State of Amazonas (VILARINHO, 2010).

According to Shaibu and Ibrahim (2016), one of the factors that may affect crop production is the physiological quality of the seeds, which has an indirect influence on the speed and percentage of seedling emergence and final stand, or a direct influence on plant vigour. Therefore, the use of seeds of high physiological quality is paramount to increasing productivity and improving the technological level of bean cultivation (ADEBISI *et al*., 2013).

Among the stages of the seed-production process, storage plays an essential role in maintaining seed quality with no deterioration, for later planting. Of the factors that determine the maximum storage potential of seeds, most noteworthy are the container, temperature, humidity and genetic characteristics of the stored product (ZUCARELI *et al*., 2015). According to Aghamir *et al*. (2016), storing seeds under controlled conditions of temperature and relative humidity allows them to be kept for long periods.

In a study conducted by Maia *et al*. (2011) to obtain information on the genetic variability associated with germination and emergence among white bean lines, the authors found that germinative power and speed of emergence decreased with storage time in environments of uncontrolled temperature and humidity. Zucareli *et al*. (2015) obtained a similar result, however they found that the physiological quality of pinto beans was reduced during storage, but at a higher rate under uncontrolled conditions of temperature and humidity.

For the cowpea, seeds are usually stored under uncontrolled environmental conditions, where the temperature, and relative humidity, together with factors inherent to the seed itself, such as water content and previous history, determine longevity (SMIDERLE *et al*., 2009).

According to Silva *et al*. (2014), there are bean cultivars of differing ability to maintain physiological quality during storage under uncontrolled environmental conditions. It is worth noting that seed quality cannot be improved during storage, but can be maintained when conditions are favourable to conservation (OLIVEIRA *et al*., 2016).

In view of the above, the aim of this study was to evaluate the physiological quality of the seeds of four cowpea cultivars produced in the State of Roraima, for different periods of storage.

## MATERIAL AND METHODS

The research was carried out from December 2012 to June 2013 at the Seed Analysis Laboratory (SAL) of Embrapa Roraima, in Boa Vista, Roraima, Brazil. The seeds of four cowpea cultivars, BRS Mazagão, BRS Guariba, UFRR Grão Verde and Pretinho Precoce 1 were used, produced in the experimental area of the Centre for Agrarian Science of the Federal University of Roraima (CAS/UFRR), Boa Vista, RR.

The seeds under evaluation came from a crop planted on 15 September 2012, intercropped with cassava (*Manihot esculenta*, Euphorbiaceae), and grown in a system of double rows (2 m x 0.5 m x 0.5 m), giving a stand of 10 plants per metre. When planting, furrows spaced 0.5 m apart were employed between two double-row sets of cassava (*M. esculenta*), using mineral fertiliser with the application of 240 kg $ha^{-1}$ of 10-10-10 formula (NPK), and the addition of half a litre of cattle manure and rice husks per metre of furrow, equivalent to 6.0 $m^3$ $ha^{-1}$. The seeds were harvested, stripped and cleaned by hand, and packed in PET (Polyethylene Terephthalate) bottles and stored for nine months starting from the first half of December 2012, under controlled conditions of temperature (20 ºC) and relative humidity (60%) in the SAL of Embrapa Roraima. The experimental design was completely randomised in a 4 x 4 factorial scheme (four cultivars and four periods of storage) with eight replications. The physical and physiological quality of the seeds was determined for four storage periods (at the start of storage and after 3, 6 and 9 months), when the following variables were evaluated:

**Germination test (G):** carried out on five replications of 50 seeds, giving a total of 250 seeds for each treatment, maintained at 25 °C and evaluated after seven days, with the results expressed as a percentage.

**First germination count (FGC):** carried out together with the germination test, calculating the percentage of normal seedlings at four days after sowing.

**Thousand-seed weight (TSW):** carried out on five replications of 100 pure seeds from each treatment, with the results expressed in grams. Finally, the appropriate approximation was applied, and the data corrected for a water content of 13%.

**Seed moisture (SM):** determined by the oven method at a temperature of 105 ± 3 °C for 24 h, in two 10-gram subsamples per treatment, with the results obtained as a percentage. The above determinations (G, FGC, TSW and SM) were made following the Rules for Seed Analysis (BRASIL, 2009).

**Electrical conductivity (EC):** carried out as per Vieira and Krzyzanowski (1999), on five replications of 50 seeds per treatment, previously weighed and immersed in 75 mL of distilled water at a temperature of 25 °C for 24 hours, with the results expressed in µS $cm^{-1}g^{-1}$ seeds.

**Water gain (G-water):** carried out together with the test for electrical conductivity; after 24 hours of immersion the seeds were dried with paper towels and weighed on a precision scale, with the results expressed as a percentage of the initial values.

**Seedling field emergence (FE):** conducted as per Nakagawa (1999), on five replications of 50 seeds per treatment, planted in beds of a sandy soil with 4 to 5% clay, at a depth of 3 cm, with irrigation which depended on crop requirement. Normal plants were counted at 21 days after sowing, and the results expressed as a percentage.

**Speed of seedling field emergence (SE):** carried out as per Popinigis (1985) together with the test for seedling field emergence, taking daily counts of emerged seedlings with cotyledons completely above the ground for each replication until the number became constant.

The results were submitted to analysis of variance and regression analysis, the mean values being compared by Tukey's test at 5% probability; the data were analysed with the SISVAR statistical software (FERREIRA, 2011).

## RESULTS AND DISCUSSION

In Table 1 can be seen the mean squares and significance levels by F-test for the characteristics under evaluation, where the data showed a significant interaction between cultivar and storage period at a level of 5% probability for all the variables being studied.

There was a significant effect from the isolated factors on all the characteristics under evaluation, except for the variables percentage germination and speed of emergence for the cultivars. Low coefficients of variation, between 1.4 and 7.5%, can also be seen, showing the accuracy of the data (Table 1).

For the 1000-seed weight of the BRS Guariba and BRS Mazagão cultivars in comparison to the initial period, no change was seen at 270 days of storage. This fact shows that the polyethylene packaging at room temperature was efficient in precluding an exchange of moisture between the seed and the environment, ensuring a lower probability of deterioration due to a rise in water content.

On the other hand, for the Pretinho Precoce 1 cultivar, the 1000-seed weight varied throughout the storage period, from 164 g to 169 g (Figure 1). However, it should be remembered that in this research, comparisons were made between genetically different seeds and not between lots of the same genotype.

In general, the variables evaluated in the present study were relatively low and uniform during storage, showing that the water content could not have influenced the physiological potential of the seeds during analysis.

Initial levels for seed moisture in the four cultivars ranged from 12.0 to 12.5% (Figure 2A). According to Ataíde, Borges and Leite Filho (2016), it is important to determine the water content of the seeds at the beginning of the evaluation, so that consistent results can be obtained when evaluating physiological potential, which would allow comparisons between different materials.

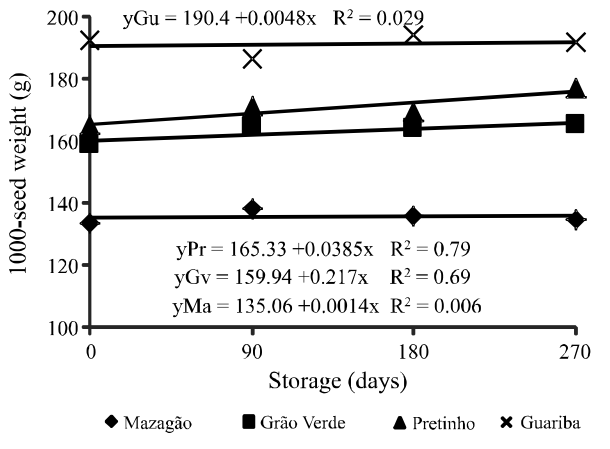
In addition to the above, the water absorption curves generated from the Pretinho Precoce 1 and BRS Mazagão cultivars tended to result in a higher percentage of imbibition in relation to the seeds of the UFRR Grão Verde and BRS Guariba cultivars, with a marked fall in the intensity of imbibition for all cultivars from 180 days, the possible end of phase I. This phase is characterised as being a physical process, since it is independent of

**Table 1 -** Summary of the variance analysis (mean squares and significance by F-test), coefficients of variation and general mean values obtained for the variables, germination (G, %), first germination count (FGC, %), plant emergence (FE, g), speed of plant emergence (SE, index), 1000-seed weight (TSW, g), electrical conductivity (EC, µS cm$^{-1}$ g$^{-1}$) and water gain (G-water, %), in the BRS Mazagão, UFRR Grão Verde, Pretinho Precoce 1 and BRS Guariba cultivars during 270 days of storage

| SV | DF | G | FGC | FE | SE |
|---|---|---|---|---|---|
| Repl. | 4 | 2.669$^{ns}$ | 23.222$^{ns}$ | 17.164$^{ns}$ | 4.393* |
| Period | 3 | 19.775** | 164.936** | 4775.77** | 278.345** |
| Cultivar | 3 | 9.150$^{ns}$ | 531.861** | 1035.09** | 2.614$^{ns}$ |
| P x C | 9 | 11.808 | 123.811** | 1932.98** | 8.459** |
| Residual | 60 | 3.705 | 11.282 | 330.127 | 1.629 |
| Total | 79 | 426.050 | 3974.49 | 8091.15 | 1034.35 |
| CV % | | 2.01 | 3.78 | 2.58 | 7.55 |
| Mean | | 95.9 | 88.7 | 72.41 | 16.908 |
| | DF | TSW | EC | G-water | |
| Repl. | 4 | 4.635$^{ns}$ | 7.105$^{ns}$ | 11.466$^{ns}$ | |
| Period | 3 | 239.749** | 131.675** | 1200.04** | |
| Cultivar | 3 | 31972.5** | 6557.34** | 469.713** | |
| P x C | 9 | 504.013** | 31.443** | 69.118** | |
| Residual | 60 | 337.669 | 9.112 | 11.668 | |
| Total | 79 | 33058.5 | 20925.2 | 6377.27 | |
| CV % | | 1.44 | 4.17 | 2.63 | |
| Mean | | 164.92 | 72.41 | 129.878 | |

** (p<0.01); * (p<0.05); $^{ns}$ (not significant)

**Figure 1 -** Mean values for 1000-seed (TSW, g) in the BRS Mazagão, UFRR Grão Verde, Pretinho Precoce 1 and BRS Guariba cultivars, at up to 270 days of storage




the metabolic activity of the seeds, and can occur both in viable or non-viable seeds (BEWLEY, BLACK, 1994). According to Forti, Cicero and Pinto (2010), the rapid gain in moisture seen in phase I in relation to the other phases is probably due to the presence of hydrophilic matrices, such as proteins. Albuquerque *et al*. (2009) describe how in this phase the first signs of metabolism reactivation appear, with a marked increase in respiratory activity and the release of energy for germination and enzyme activation.

In relation to the results of the test for electrical conductivity (Figure 2B), the superiority of the seed quality of the Pretinho Precoce 1 cultivar should be noted, presenting a lesser release of exudates in relation to the BRS Mazagão and UFRR Grão Verde cultivars, while the BRS Guariba cultivar displayed a greater release of exudates present in the aqueous solutions of the imbibed seeds for all the periods under evaluation.

According to Matos, Borges and Silva (2015) the test for electrical conductivity (EC) is based on the permeability of the cell membranes, i.e. during the loss of seed viability, there is an increase in the exudates of seed cells soaked in water, due to the loss of integrity of these membranes. The values for EC will be lower, the greater the physiological quality of the seeds (MOURA *et al*., 2016).

In the present study, the cultivars displayed mean values for germination of over 93% for all the periods of

**Figure 2** - Mean percentage values for (A) water content (%) and (B) electrical conductivity (EC, µS cm$^{-1}$ g$^{-1}$) in seeds of the BRS Mazagão, UFRR Grão Verde, Pretinho Precoce 1 and BRS Guariba cultivars, at up to 270 days of storage

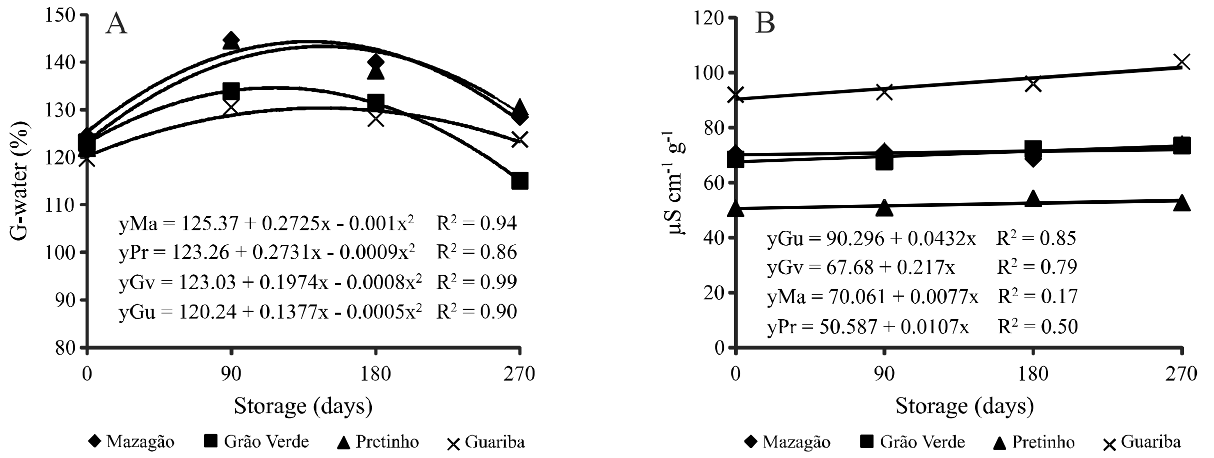
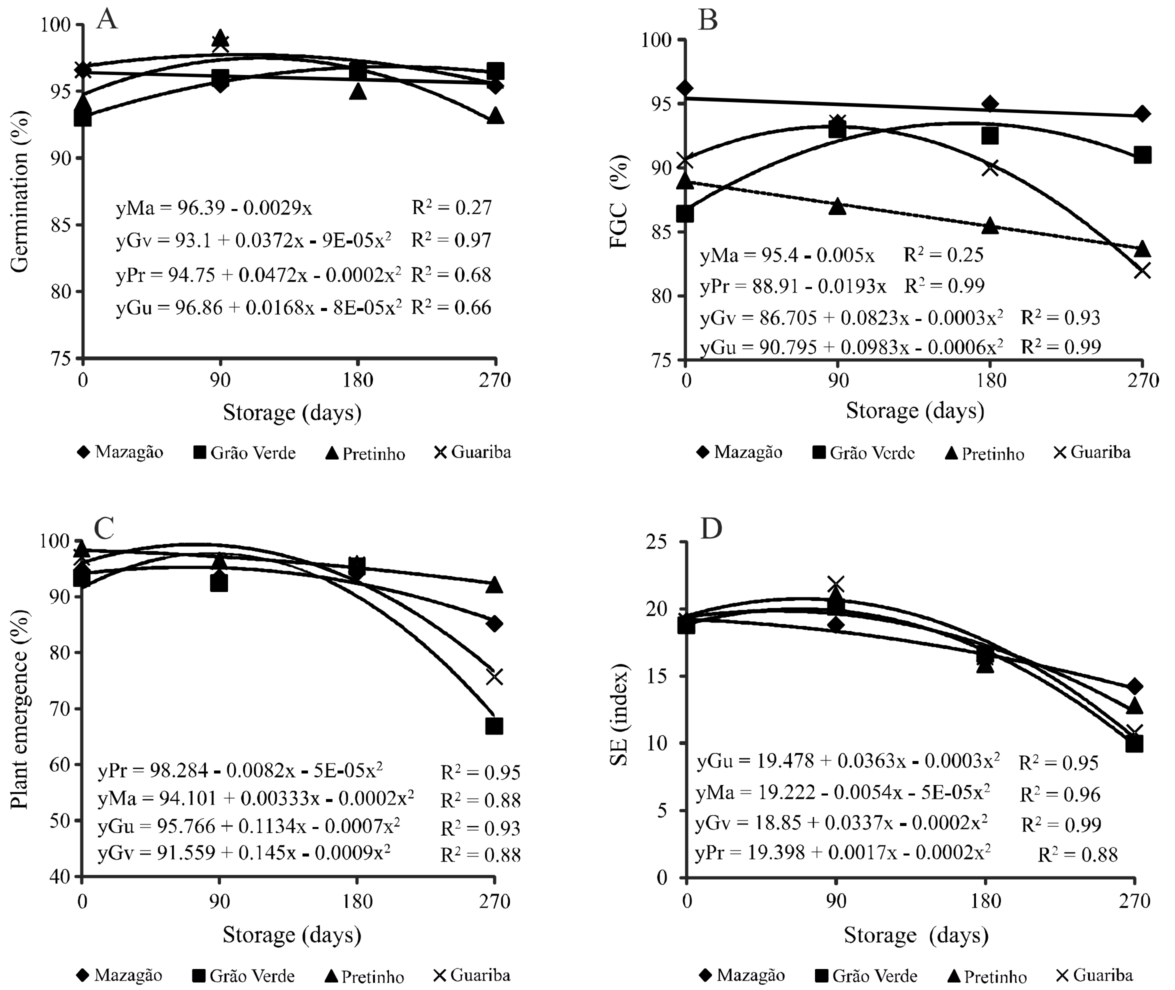



**Figure 3** - Mean values for (A) percentage germination (G, %), (B) first germination count (FGC), (C) seedling field emergence (FE) and (D) speed of seedling emergence (SE, index) in seeds of the BRS Mazagão, UFRR Grão Verde, Pretinho Precoce 1 and BRS Guariba cultivars, at up to 270 days of storage



storage (Figure 3A), while for first germination count, the BRS Guariba cultivar displayed better percentage yields at up to 90 days; after this period, there was a sharp fall (Figure 3B) compared to the UFRR Grão Verde and BRS Mazagão cultivars. On the other hand, the Pretinho Precoce 1 cultivar showed a decrease in first germination count for all the periods under evaluation (Figure 3B).

For seedling field emergence (Figure 3C), the four cultivars showed an average percentage of over 90%, for the 0-90 day period, while at 270 days it was found that the BRS Guariba and UFRR Grão Verde cultivars showed a decrease of 30% in seed vigour, compared to the BRS Mazagão and Pretinho Precoce 1 cultivars. Storage therefore affected seed quality in the BRS Guariba and UFRR Grão Verde cultivars.

At 270 days of storage, Rodrigues *et al*. (2015) working with the BRS Guariba cultivar, achieved 98% germination and 62% field emergence. This result confirms that storage has a negative effect on seed vigour in the BRS Guariba cultivar.

Silva *et al*. (2014) reported that a decline in physiological potential over time is not restricted only to a reduction in germination capacity, which becomes slower, but also to a sharpened sensitivity to environmental adversity, characterising the fall in seed vigour.

According to Martins *et al*. (2016), the test for seedling field emergence is considered a reference test, since for any test of vigour to be considered efficient in the area of seed analysis, it must present a good correlation with seedling field emergence.

Based on the results presented in the present research, it is possible to affirm that the storage potential of seeds varies considerably between cowpea cultivars. This potential is determined by the storage period, so not all cultivars will survive for the same length of time under the same conditions. For practical purposes, storage is of great benefit in commercial seeds, being of great benefit as it allows the producer to define the storage system to be used.

## CONCLUSIONS

1. Physiological quality in seeds of the BRS Guariba cultivar is influenced by the storage period;

2. Irrespective of storage period, seeds of the BRS Mazagão cultivar display superior physiological quality in relation to the other cultivars;

3. Seeds of the BRS Guariba and UFRR Grão Verde cultivars show a reduction in physiological quality after three months of storage.

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