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Effect of drying conditions on physiological quality of cowpea seeds of the cultivar brs-tumucumaque

Condições de secagem na qualidade fisiológica de sementes de feijão-caupi, cv. brs tumucumaque

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ABSTRACT - Seed drying has many advantages, but it can cause irreversible damages, compromising the physiological quality of seeds, especially when they present high water contents. The objective of this work was to evaluate the effect of drying conditions (inside and outside the pod) on the physiological quality of cowpea seeds of the cultivar BRS-Tumucumaque before and after storage. A completely randomized experimental design with four replications was used, in a split-plot arrangement. The plots consisted of combinations of harvest seasons (1, 2, 3, 4, and 5) and artificial seed drying conditions (inside and outside the pod); and the subplots consisted of storage times (0 and 6 months). The seed water contents were determined and the seeds were evaluated for germination, first germination count, emergence, emergence index, accelerated aging, and electrical conductivity. Higher physiological quality was found for dried seeds inside the pods, which was more evident in seasons 1 and 2, in both storage times. In the other seasons, the drying conditions had no effect on seed germination and vigor at the beginning of storage. The dried seeds inside the pods showed greater vigor after six months of storage. Drying seeds inside the pods favors the maintenance of physiological quality and allows artificial drying of cowpea seeds of the cultivar BRS-Tumucumaque with water content of 47%. Artificial drying of seeds with water contents higher than 32.5% is not recommended for the condition outside the pod. The seed physiological potential decreases after six months of storage, regardless of the drying condition.

Keywords: *Vigna unguiculata*. Germination. Seed vigor. Artificial drying. Storage.

RESUMO - A secagem pode causar prejuízos à qualidade das sementes, principalmente quando apresentam teor de água elevado. Objetivou-se avaliar a influência de condições de secagem na qualidade fisiológica inicial e após armazenamento de sementes de feijão-caupi, cv. BRS Tumucumaque. O delineamento experimental utilizado foi inteiramente casualizado, com quatro repetições, em esquema de parcelas subdivididas. Nas parcelas foram alocadas a combinações dos fatores época de colheita (1, 2, 3, 4 e 5) e condição de secagem artificial das sementes (dentro e fora da vagem); os tempos de armazenamento (0 e 6 meses) foram alocados na subparcela. Determinou-se o teor de água e as sementes foram avaliadas pelos testes de germinação, primeira contagem de germinação, emergência, índice de velocidade de emergência, envelhecimento acelerado e condutividade elétrica. Resultados superiores de qualidade fisiológica foram verificados para as sementes secas dentro da vagem, fato mais evidente nas épocas 1 e 2, em ambos os tempos de armazenamento. Para as demais épocas, no início do armazenamento não houve efeito da condição de secagem na germinação e vigor. As sementes secas dentro da vagem apresentaram maior vigor após seis meses de armazenamento. A secagem dentro da vagem favorece a manutenção da qualidade fisiológica e permite a secagem artificial de sementes de feijão-caupi, cv. BRS Tumucumaque, com teor de água de 47%. Não se recomenda a secagem artificial de sementes com teor de água acima de 32,5% na condição fora da vagem. Após seis meses de armazenamento, independentemente da condição de secagem, há redução no potencial fisiológico das sementes.

Palavras-chave: *Vigna unguiculata*. Germinação. Vigor de sementes. Secagem artificial. Armazenamento.

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INTRODUCTION

Seeds are an important input in agriculture and the subject for many advances in plant genetics, since it is responsible for the establishment of the plant stand in the field (ANAND et al., 2019). Seeds should be harvested at the physiological maturity point, when they have maximum physiological potential. However, uneven maturation makes the determination of this moment difficult and results in a high predisposition to the occurrence of injuries in seeds due to their high-water contents.

Drying operations have contributed to seed quality in seed producing companies because it enables to anticipate the harvest and the maintenance of the initial seed quality during storage. However, a careful planning and execution of the drying process is necessary, especially when the harvested seeds have high water contents, because the vapor pressure gradient between their interior and the drying air may increase during the drying process. It results in high drying rates, i.e., high percentage of water removed in a shorter period, and high probability of occurrence of injuries to cell structures and, consequently, in quality loss (AMARO et al., 2021; QUEQUETO et al., 2020).

The physiological results of high drying rates are shown by the reduced germination and germination speed (OBA et al., 2019); increased seedling abnormality rate (HARTMANN FILHO et al., 2016); and decreased conservation potential during storage; however, these losses may not show immediately. The physiological quality of seeds during storage can reduce significantly, as found for rice (SCARIOT et al., 2021), sunflower (HUANG et al., 2021), corn (VERGARA et al., 2018), and safflower (OBA et al., 2019).

Drying inside the pod or using artificially heated temperatures was favorable against rapid desiccation for soybean (SAMARAH et al., 2009) and common bean seeds (ARAÚJO et al., 1984).

However, studies on drying procedures for cowpea seeds have only approached aspects related to drying kinetics (MABASSO et al., 2022; CAMICIA et al., 2015; MORAIS et al., 2013). Therefore, the objective of this work was to evaluate the effect of drying conditions (inside and outside the pod) on the physiological quality of cowpea seeds of the cultivar BRS-Tumucumaque before and after storage.

MATERIAL AND METHODS

The experiment was conducted in field and laboratory conditions from February to November 2018. Cowpea seeds were sown in February 2018 in a no-till system using a seeder-fertilizer, distributing 10 seeds per meter to depths of 3 to 4 cm. The cowpea cultivar used was the BRS-Tumucumaque, which has a semi-upright architecture and indeterminate growth habit (OLIVEIRA et al., 2014). The soil fertilization at planting was based on the soil chemical analysis, using 150 kg ha^{-1} of the 08-28-16 N-P-K formulation. The planted area was 1000 m^2.

Rainfall depths, maximum, mean, and minimum temperatures, and mean relative air humidity were monitored (Figure 1). Climatological records were obtained from a station of the National Institute of Meteorology (INMET) installed in the Federal University of Viçosa, in Viçosa, MG Brazil.

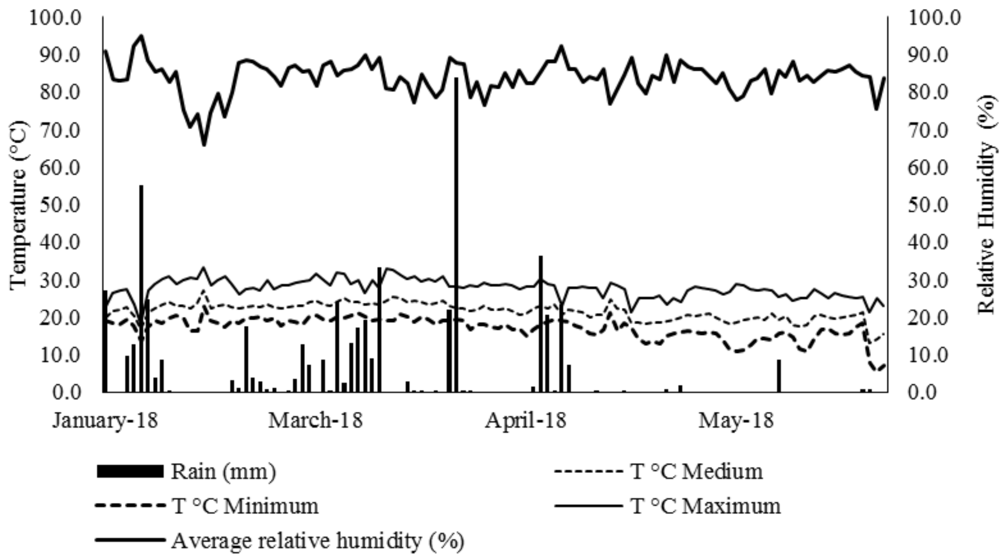

Figure 1. Daily data of rainfall depth, maximum, mean, and minimum temperatures, and mean relative air humidity collected during the period of the experiment. Source: INMET (2018).

The pods were harvested by hand-picking the plants at 30 days, after 50% of the flowers were opened, which characterizes the full flowering stage. The harvests continued with regular intervals of eight days until the whole field presented 100% of the pods with a yellow-brown color (Figure 2C).

The pods were randomly harvested, collecting six single samples of the same size (60 plants) at different points, walking in zigzag in the field, with entry and exit in different locations. Thus, all parts of the field were sampled.

The six single samples collected were combined, forming a composite sample of 360 plants, which were homogenized, composing a representative sample of the seed harvest season.

After each harvest, the composite samples of cowpea plants were sent to the Seed Research Laboratory of the Agronomy Department of the Federal University of Viçosa (UFV). The pods were detached from the plants and a seed sample was used for determining water contents at the harvest time using the oven method (105±3 °C for 24 hours) (BRASIL, 2009). The pods were then visually classified according to their color at harvest as green, purple, and yellow-brown (Figures 2 A, B, and C).

Five harvests were carried out (Figure 3) considering the percentage of pods of each color and seed water contents at harvest. Seeds from the harvest seasons 1, 2, 3, 4, and 5 had water contents of 57%, 47%, 32.5%, 27.7%, and 24%, respectively (Figure 3).

Figure 2. Maturity stages of cowpea seeds of the cultivar BRS-Tumucumaque defined based on the color of the pods: green pods (A), purple pods (B), and yellow-brown pods (C).

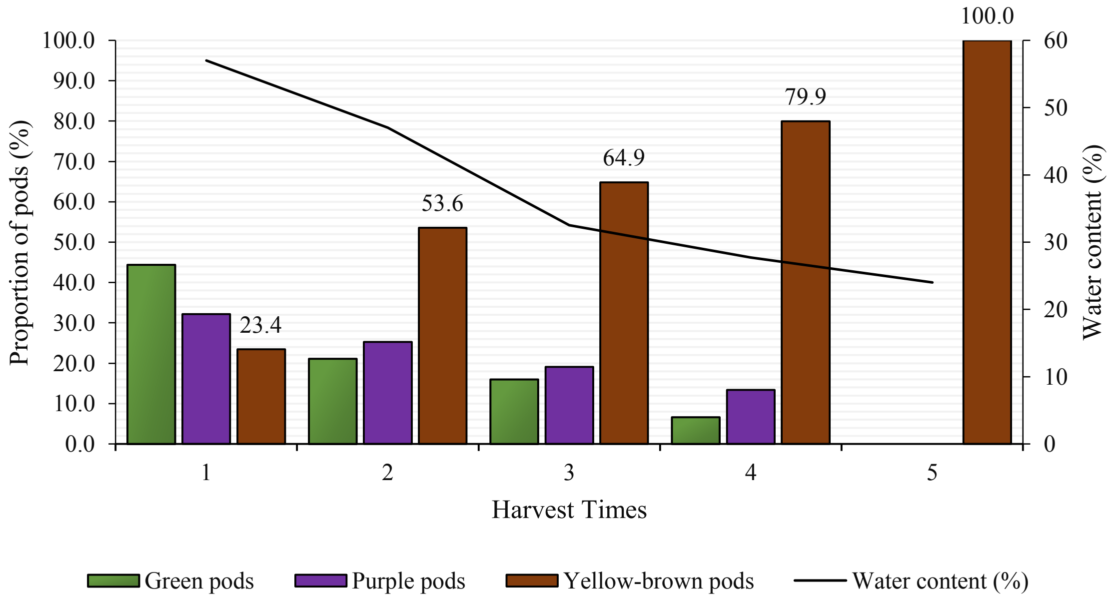

Figure 3. Proportion of green, purple, and yellow-brown pods and water contents of cowpea seeds of the cultivar BRS-Tumucumaque at harvest.

The drying process of half of the harvested pods was completed with seeds inside the pods in each harvest season, until they were dry and easy to thresh. The other half was manually threshed and the drying process was completed outside the pod. The seeds were dried in a forced air circulation oven set at 30 °C with mean relative air humidity of 60% until they reached a mean water content of approximately 13%.

The water loss of the seeds was monitored by periodic weighing using the equation proposed by Cromarty, Ellis and Roberts (1985):

$$Mi(100-Ui) = Mf(100-Uf)$$

Where:
Mi = initial seed weight (g);
Ui = initial seed water content (%);
Mf = final seed weight after drying (g); and
Uf = final seed water content after drying (%).

The seeds were then cleaned and manually processed through a set of metal sieves with oblong holes of six dimensions: 14/64 × 3/4, 13/64 × 3/4, 12/64 × 3/4, 11/64 × 3/4, 10/64 × 3/4, 9/64 × 3/4.

The physiological quality of the seeds was evaluated using the ones retained on sieves with dimensions of 13/64 × 3/4, 12/64 × 3/4, and 11/64 × 3/4, as they presented a higher percentage of water and uniformity. Seeds with unsatisfactory qualities for commercialization, such as those that were immature, malformed, shocked, deformed, or attacked by pathogens or insects, were discarded.

Subsequently, the following initial quality tests were performed:

Germination: four replications of 50 seeds were germinated on paper towel rolls moistened with distilled water at the rate of 2.5-fold the dry substrate weight. The rolls were then placed in plastic bags and kept in a germination chamber at a constant temperature of 30 °C. Normal seedlings were counted at five and eight days after the beginning of the test and the results were expressed as percentages of normal seedlings (BRASIL, 2009).

Emergence: evaluations were conducted in an acclimatized growing room at a constant temperature of 25 °C with four replications of 50 seeds. The seeds were sown to a depth of three centimeters in expanded polystyrene trays containing washed sterilized sand. Irrigation was done with sprinklers when substrate wetting was needed. The emerged seedlings were counted on the eighth day after sowing. Seedlings with cotyledons above the substrate were counted. The results were expressed as percentages.

Emergence speed index: determined together with the emergence test. Seedlings with cotyledons above the substrate were daily counted until stabilization. The germination speed index of the seedlings was calculated according to Maguire (1962).

Accelerated aging: four replications of 50 seeds were distributed on a suspended screen inside a transparent acrylic box (11 × 11 × 3.0 cm) containing 40 mL of deionized water. The boxes were kept in a germination chamber set to 42 °C for 72 hours (DUTRA; TEOFILO, 2007). The seeds were then germinated as described in the germination test. The seedlings were evaluated on the fifth day after sowing and the results were expressed as percentages of normal seedlings.

Electrical conductivity: four replications of 50 seeds were weighed with precision of 0.01 g and then soaked in 75 mL of deionized water in 200-mL plastic cups for 24 hours at 25 °C in a germination chamber (DUTRA; MEDEIROS FILHO; TEÓFILO, 2006). The electrical conductivity of the soaking solution was then measured using a Digimed conductivity meter (model DM 31); the results were expressed in µS cm^{-1} g^{-1} of seed.

After the initial evaluation, the seeds were packed in permeable Kraft paper packages and stored for six months in a room with natural air circulation. The water content and quality evaluations were then performed again as described above.

The temperature (maximum, mean, and minimum) and mean relative air humidity over the storage times were monitored daily using a data logger. The mean temperature and relative humidity during the storage times were 22.7 °C and 65.5%, respectively. The highest temperature recorded was 32.9 °C and the minimum was 16.2 °C.

The experiment was conducted in a completely randomized experimental design with four replications, in a split-plot arrangement. The plots consisted of combinations of harvest seasons (1, 2, 3, 4, and 5) and artificial seed drying conditions (inside and outside the pod); and the subplots consisted of storage times (0 and 6 months).

The data were subjected to analysis of variance after checking for normality of errors by the Shapiro-Wilk test and for homogeneity of variance by the Oneill and Mathews test at significance of $p < 0.05$. The effects of the harvest seasons were grouped by the Scott-Knott test at 5% probability level. The F test was used to compare the drying conditions and storage times.

RESULTS AND DISCUSSIONS

The interaction between harvest seasons, drying conditions, and storage times was not significant for the emergence speed index (ESI). However, the interaction between harvest seasons and drying conditions was significant for this variable.

The seed water contents before and after the storage time (six months) were between 11.1% and 12.4% (Table 1). Despite not being controlled, the storage environment allowed the water content to remain at acceptable levels for storage, because it was kept dry and ventilated. In addition, the packages had no direct contact with the floor, walls, or sunlight.

Table 1. Water contents (%) of cowpea seeds of the cultivar BRS-Tumucumaque subjected to drying (inside and outside the pods) and storage (initial and six months).

Harvest season	Drying	Storage	
		Initial	Six months
E1	Inside the pod	12.6	12.0
E1	Outside the pod	12.9	11.1
E2	Inside the pod	12.7	12.5
E2	Outside the pod	12.7	12.5
E3	Inside the pod	12.8	12.0
E3	Outside the pod	12.3	11.9
E4	Inside the pod	12.8	12.4
E4	Outside the pod	12.7	11.3
E5	Inside the pod	12.4	11.8
E5	Outside the pod	12.7	11.8

E1 = harvest season 1; E2 = harvest season 2; E3 = harvest season 3; E4 = harvest season 4; E5 = harvest season 5.

Storage time: initial (0 months)

The unfolding of the data of harvest periods within the drying condition inside the pod showed that the seeds from harvest period 5 (E5) resulted in lower germination and first germination count than the other harvest periods, which were similar to each other (Table 2).

Harvest seasons 1 (E1), 2 (E2), and 4 (E4) had higher emergence than the harvest seasons 3 (E3) and E5. E1 and E3 presented higher seed vigor in the accelerated aging test. Lower electrical conductivity values were found for E1 and E2 when compared to the other seasons (Table 2). Regarding the emergence speed index, the drying of seeds inside the pod resulted in greater seed vigor in harvest seasons E1, E2, and E4 when compared to E3 and E5 (Table 3).

The drying of seeds inside the pod probably favored the maintenance of the initial physiological quality, especially for E1 and E2. These harvest seasons had high proportion of green pods and, consequently, larger amounts of immature seeds, and higher water contents (57% and 47%, respectively), thus, it was presumed that immediate damage would be caused by drying. However, the physical presence of the pod may have acted as a natural barrier against rapid water loss to the environment. Furthermore, protective cellular mechanisms may have been activated due to the more gradual loss of water. In this drying condition, the enzymatic activity and the modification of proteins are maintained, continuing the maturation process. These cellular mechanisms, such as LEA-like proteins and oligosaccharides, increase the resistance to drastic dehydration (LEPRINCE et al., 2017).

Table 2. Germination, first germination count, emergence, accelerated aging, and electrical conductivity of cowpea seeds of the cultivar BRS-Tumucumaque, according to harvest seasons, drying conditions (inside and outside the pod), and storage times (0 and 6 months).

Harvest Seasons	Initial		Six months	
	Drying inside the pod	Drying outside the pod	Drying inside the pod	Drying outside the pod
	Germination (%)			
E1	92.0 Aa (A)	75.0 Bb (A)	55.0 Ac (B)	35.0 Bc (B)
E2	90.0 Aa (A)	81.0 Ba (A)	75.0 Aa (B)	60.0 Bb (B)
E3	93.0 Aa (A)	84.0 Ba (A)	67.0 Ab (B)	69.0 Aa (B)
E4	89.0 Aa (A)	65.0 Bc (A)	62.0 Ac (B)	54.0 Bb (B)
E5	68.0 Ab (A)	52.0 Bd (A)	59.0 Ac (B)	54.0 Ab (A)
	First germination count (%)			
E1	88.0 Aa (A)	73.0 Ba (A)	51.0 Ab (B)	31.0 Bc (B)
E2	86.0 Aa (A)	78.0 Ba (A)	65.0 Aa (B)	54.0 Ba (B)
E3	87.0 Aa (A)	78.0 Ba (A)	56.0 Ab (B)	58.0 Aa (B)
E4	87.0 Aa (A)	58.0 Bb (A)	53.0 Ab (B)	47.0 Ab (B)
E5	63.0 Ab (A)	49.0 Bc (A)	43.0 Ac (B)	45.0 Ab (A)
	Emergence (%)			
E1	97.0 Aa (A)	81.0 Bb (A)	95.0 Aa (A)	65.0 Bc (B)
E2	97.0 Aa (A)	83.0 Bb (A)	80.0 Ac (B)	81.0 Ab (A)
E3	91.0 Ab (A)	93.0 Aa (A)	79.0 Bc (B)	87.0 Aa (A)
E4	93.0 Aa (A)	92.0 Aa (A)	88.0 Ab (A)	88.0 Aa (A)
E5	85.0 Ab (A)	79.0 Ab (A)	81.0 Ac (A)	81.0 Ab (A)
	Accelerated aging (%)			
E1	73.0 Aa (A)	28.0 Bd (A)	44.0 Ab (B)	12.0 Bc (B)
E2	66.0 Ab (A)	49.0 Bb (A)	65.0 Aa (A)	23.0 Bb (B)
E3	74.0 Aa (A)	77.0 Aa (A)	38.0 Bb (B)	61.0 Aa (B)
E4	49.0 Bc (A)	74.0 Aa (A)	6.0 Bc (B)	14.0 Ac (B)
E5	30.0 Bd (B)	41.0 Ac (A)	41.0 Ab (A)	0.0 Bd (B)
	Electrical conductivity (µS cm^{-1} g^{-1})			
E1	93.1 Aa (A)	148.2 Bd (A)	101.1 Ab (B)	147.3 Bd (A)
E2	92.0 Aa (A)	132.7 Bc (B)	87.4 Aa (A)	112.5 Bb (A)
E3	108.2 Ab (B)	102.5 Aa (B)	96.0 Aa (A)	90.3 Aa (A)
E4	116.3 Ab (B)	112.2 Ab (A)	96.4 Ab (A)	107.1 Bb (A)
E5	112.1 Ab (A)	137.3 Bc (A)	111.3 Ac (A)	132.3 Bc (A)

Means followed by the same lowercase letter are part of the same group by the Scott-Knott test at 5% probability. Means followed by the same capital letter in the rows, comparing drying conditions (inside and outside the pod) in each storage time are not different from each other by the F test ($P < 0.05$).
Means followed by the same uppercase letter within parenthesis, comparing storage times (initial and 6 months) in each drying condition are not different from each other by the F test ($P < 0.05$). E1 = harvest season 1; E2 = harvest season 2; E3 = harvest season 3; E4 = harvest season 4; E5 = harvest season 5.

Table 3. Emergence speed index of cowpea seeds of the cultivar BRS-Tumucumaque subjected to two drying conditions (inside and outside the pod).

Emergence speed index					
	Harvest season				
Drying condition	E1	E2	E3	E4	E5
Inside the pod	10.9 aA	10.9 aA	10.3 bA	11.3 aA	10.1 bA
Outside the pod	8.8 cB	9.6 bB	10.8 aA	11.3 aA	9.6 bA

Means followed by the same lowercase letter in the rows are part of the same group by the Scott-Knott test at 5% probability. Means followed by the same uppercase letter in the columns are not different from each other by the F test ($P < 0.05$). E1 = harvest season 1; E2 = harvest season 2; E3 = harvest season 3; E4 = harvest season 4; E5 = harvest season 5.

In the drying condition outside the pod, differently from what was observed previously, seeds from seasons E1 and E2 exhibited low vigor, as shown by the accelerated aging and electrical conductivity tests (Table 2) and emergence speed index (Table 3).

Seeds may present physical changes during the drying process due to temperature and humidity gradients, which cause cell expansion, contraction, and changes in density and porosity (ARAUJO et al., 2014; MUNDER; ARGYROPOULOS; MÜLLER, 2017; RODRIGUES et al., 2020). Seeds with high water contents, as those in E1 (57%) and E2 (47%), may show increases in vapor pressure gradient between their interior and the drying air, resulting in high drying rates (OLIVEIRA et al., 2016).

The seed stress due to high water contents during this process can cause injuries, such as cracking, fissures, and damage to cellular structures (MENEZES et al., 2012). It may disorganize the cell membrane system, making the membranes lose their effectiveness as leaching barriers during the soaking initial stages. It can be indirectly detected by the electrical conductivity test, in which the harvested seeds in E1 and E2 and dried outside the pod presented higher electrical conductivity and, thus, low vigor (Table 2).

The seed deterioration process is accelerated due to the disorganization of cell membranes, affecting the seed germination and vigor, mainly by increasing the leaching of essential constituents for germination, and loss of cell compartmentalization and repair mechanisms (ZHANG et al., 2021).

The effect of drying conditions at each harvest season showed that the drying inside the pod resulted in higher physiological quality for E1 and E2, compared to the drying outside the pod (Table 2). The other seasons presented, in general, no effect of the drying condition on the seed physiological quality (Table 2). Most seeds probably had already some efficient desiccation protection mechanism, which allowed them to maintain their viability even after artificial drying at a temperature of 30 °C.

Storage time: 6 months

The unfolding of the data of harvest seasons within the drying condition inside the pod showed that seeds in E2 presented, in general, higher physiological quality. This result reinforces the importance of keeping the pod during the drying process of seeds with high water content (47%), as shown in the initial evaluation, and harvesting seeds as close as possible to their physiological maturity.

Water content can be used as an indicator of the moment that seeds reach physiological maturity. However, a rapid dehydration starts after this moment. Nogueira et al. (2014) defined the moment between 14 and 18 days after anthesis, when the seed water contents went from 53.6% to 14%, as the physiological maturity point, to evaluate the development and physiological quality of cowpea seeds of the cowpea cultivar BRS-Guariba during the maturation process. Cruz et al. (2019) reported that seeds of the cowpea cultivar Corujinha reached physiological maturity at 15 days after anthesis, when the water content exceeded 80%. In the present study, the seed water content in E2 (47%) was below this value. The harvesting in E2 probably favored the obtaining better quality seeds, since they remained in the field for less time under adverse conditions, which can accelerate the deterioration process when compared to later harvests.

However, despite the seed water content of 57%, the physiological quality of seeds harvested in E1 were, in general, similar that obtained in E2. In this case, the latent damage of drying seems to have been more deleterious to the seed physiological quality, since many seeds harvested in E1 had not yet completed the maturation process. Therefore, they possibly had not yet acquired the ability to tolerate desiccation. The molecular basis of tolerance is the accumulation of protectors, such as LEA proteins, which stabilize the structure of proteins and cell membranes, especially during cellular dehydration (AMARA et al., 2014).

In the other harvest seasons, in addition to the latent effect of drying on seed quality, the delay in removing them from the field may also have had negatively affected their conservation potential. There was a difference of 32 days between harvests E1 and E5. Thus, there were 32 days of exposure to adverse conditions for pods turn to yellow-brown and the seeds complete their maturation process, which may have accelerated the deterioration process and compromised the physiological potential.

Contrastingly, when considering the harvest seasons within the drying condition outside the pod, seeds harvested in E3 presented the highest physiological quality. Many seeds from E3 probably had already an efficient desiccation protection mechanism, which prevented the disorganization of membranes and ensured the replacement of water necessary for the maintenance of membrane structure (LEPRINCE et al., 2017).

In the same analysis, seeds harvested in E1 were more affected by the artificial drying (Table 2), due to the greater susceptibility of this treatment to the occurrence of injuries that affect the cell membrane and accelerate the deterioration process. Cellular ruptures can promote the leakage of cellular contents (enzymes, proteins, amino acids, carbohydrates, lipids, ions, etc.), causing a chain of undesirable and irreversible reactions (CORADI et al., 2016).

The effect of drying conditions within each harvesting season showed that drying inside the pod resulted in higher physiological quality for E1 and E2. It reinforces the importance of the physical performance of the pod as a natural barrier against rapid water loss of seeds with high water contents at the time of drying, not only in terms of initial quality but also in terms of potential for conservation. In the other seasons, in general, the presence of the pod did not interfere with the quality results.

CONCLUSIONS

Drying inside the pod favors the maintenance of physiological quality and allows the artificial drying of cowpea seeds of the cultivar BRS-Tumucumaque with 47%

water content.

Artificial drying of seeds with water content above 32.5% in the condition outside the pod is detrimental to germination and vigor of cowpea seeds of the cultivar BRS-Tumucumaque.

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