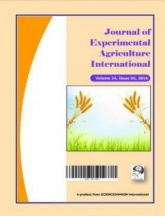

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Moisture Content and Packaging Condition on the Germination of Amaranth BRS Alegria Seeds

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Authors' contributions

This work was carried out in collaboration among all authors. Author PMCC designed the study, performed the statistical analysis, wrote the first draft of the manuscript and managed the literature searches. Authors AB and CC managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

The objective of this work was to evaluate the effect of the packaging conditions and the initial moisture content of amaranth BRS Alegria seeds, stored in the period of 13 months (10 months ambient conditions and 3 months in cold chamber). The experimental design was completely randomized in a factorial scheme with two initial moisture contents (9.82% and 7.84%), and two packing conditions (sealed and unsealed), with four replications, totaling 16 plots. The germination was in 12 hours photoperiod and 25°C constant temperature, for 7 days. The variables analyzed were percentage of germination (PG), first germination count (FGC), non-germinated seeds (NGS), germination speed index (GSI) and mean germination time (MGT). The sealed 9.82% seed lot presented better results for PG, NGS and GSI (77.5%, 22.5% and 24.88, respectively). The

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moisture content of 9.82% is adequate for the storage of *Amaranthus cruentus* BRS Alegria, with no further drying required. It is recommended to seal the packages for the storage of amaranth seeds of BRS Alegria, as it maintains its physiological quality for longer.

Keywords: Amaranthus cruentus; first germination count; germination speed index; mean germination time.

1. INTRODUCTION

The Amaranth (*Amaranthus cruentus* L.) is one of the species that has good grain production and has rapid growth [1]. It is rich in proteins, compared to most cereals, and of high biological value, with a high content of lysine, methionine and sulfur amino acids, limiting in other cereals and legumes [2,3,4,5,6]. Due to its agronomic characteristics and easy adaptation, the potential of amaranth to the Brazilian savanna, increasing the income in the agricultural productive chain is great [1,7]. The BRS Alegria cultivar is a national variety selected for its adaptation to savanna climate [1].

The reduction of seed moisture contents is necessary to maintain the physiological quality of seeds during storage and this is done through drying. In general, amaranth grains are naturally dried through exposure to ambient air [8,9,10]. However, in large-scale crops, artificial drying is necessary and this can cost [9,11,12,13].

The ability of a seed to maintain its quality during storage depends on the longevity inherent to the species, its initial quality and environmental storage conditions [14]. Thus, the seed can be grown under a rigorous system, have adequate harvest, and processed to the highest purity, but can be lost if stored under inadequate conditions.

The physiological quality of stored seeds may be related to the type and packaging conditions used [15]. When the seeds are stored in permeable packages, their moisture content varies according to the variations of the humidity of the air and in semipermeable packages there is some resistance to the exchanges, but nothing that completely prevents the passage of moisture [16]. The permeability of the packages can also relate to their own sealing. Polyethylene packages, when violated, have characteristics of permeable packages, while when properly sealed, they are semipermeable [15].

The BRS Alegria cultivar with 10% moisture content can be safely stored for ten months of cold storage. At 11.7% moisture content, stored at higher temperatures, its physiological quality is impaired [17].

Despite advances in amaranth research, little information related to the seed storage process is found [17]. In this context, the present study aimed to evaluate the effect of the packaging conditions and the initial moisture content of amaranth BRS Alegria seeds, stored in the period of 13 months (10 months ambient conditions and 3 months in cold chamber).

2. MATERIALS AND METHODS

The work was carried out in the Laboratory of Seeds of the Faculty of Agronomy and Animal Science of the Federal University of Mato Grosso. Seeds of *Amaranthus cruentus* L. BRS Alegria cultivar were used from plants grown at the Rio Verde Technological Research and Development Foundation, MT 449, Km 8, in the Lucas do Rio Verde city, Mato Grosso state, geographic coordinates 13°00'27 "S and 55°58'07"W, with an average elevation of 387 meters. The climate is tropical (Aw), according to the classification of Köppen [18], and the predominant soil is dystrophic Red-Yellow Latosol of clay texture, and flat relief.

The harvest was done manually on June 06 2017, with a mean moisture content of 22%. The panicles being carefully removed to avoid grain dropping, and then the panicles were threshed by a mechanical thresher and the seeds was dried in the sun in a thin layer under until they reaching approximately 12% moisture, as recommended by Spehar et al. [1]. Then, the seeds were beneficiated to remove impurities, passing them in hot air blower with counterflow at the mean velocity of 4.5 ms^{-1} and air temperature of 30°C, obtaining clean seeds with moisture content of 9.82%. A portion of the seeds returned to the drying process until the mean moisture content of 7.84% was obtained (Table 1). Seed moisture was determined according to the Rules for Seed Analysis (105 ± 3°C for 24 hours) [19].

Table 1. Pre-test results of amaranth BRS Alegria seeds germination stored for 10 months

Moisture content	Variables				
	PG (%)	FGC (%)	NGS (%)	GSI	MGT (days)
9.82%	69.33	69.33	30.67	17.33	2.05
7.84%	62.00	60.00	38.00	15.42	2.07
Mean	65.67	64.67	34.33	16.38	2.06

PG: percentage of germination; FGC: first germination count; NGS: non-germinated seeds; GSI: germination speed index; and MGT: mean germination time

The lots were stored in sealed polyethylene packages for 10 months and kept in a laboratory subject to ambient conditions. In this period, the maximum and minimum temperatures of 33.8°C and 22.1°C, and maximum and minimum relative humidity of 76% and 22%, respectively, were obtained with a thermohygrometer model 303C. After this period the lots characterization was done, using four replicates (Table 1).

The germination test was carried out at 25°C constant temperature and 12 hours photoperiod, as recommended by Crivelari Costa and Bianchini [20] for amaranth BRS Alegria seeds in Biochemical Oxygen Demand (B.O.D.) incubator during seven days. 50 sanitized seeds were placed to germinate in transparent plastic boxes (11.0 x 11.0 x 3.5 cm) containing two sheets of blotting paper, moistened with amount of distilled moisture equivalent to 2.5 times the mass of the dry substrate. Seed sanitization was done with 0.5% sodium hypochlorite solution (NaClO) for one minute to eliminate possible contaminations and it was washed with running moisture to avoid the effect of the product according to the Manual of Sanitary Seed Analysis [21].

The variables analyzed were: percentage of germination (PG), first germination count (FGC), non-germinated seeds (NGS), germination speed index (GSI) and mean germination time (MGT).

Healthy seeds with protrusion of the primary root with 1.0 mm length or more were considered to be germinated. The PG and NGS were counted on the seventh day, when germination stabilization was observed. The FGC was counted on the third day [19]. The first germination count was performed in order to have a sense of the vigor of these seeds, since this test can detect possible differences in the physiological quality of seed lots that have similar germinative power, but may exhibit different behaviors after storage [22].

As with the first germination count, the germination speed index is also indicative of seed vigor, and may also exhibit good sensitivity in seed lots [22].

The germination speed index was calculated with the germination test and calculated according to Maguire [23], using the formula presented in Equation 1:

$$GSI = \sum_{i=1}^{N} \left(\frac{G_i}{N_i}\right) \quad (1)$$

Where: GSI is the germination speed index, dimensionless; G_1, G_2 and G_i is the number of germinated seeds in the first count, second count, ... i-th counting, respectively, dimensionless; and N_1, N_2 and N_i is the number of sowing days at the first count, second count, ... i-th counting, respectively, dimensionless.

The mean germination time was calculated using the expression proposed by Labouriau [24] presented in Equation 2:

$$MGT = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i} \quad (2)$$

Where: MGT is the mean germination time, in days; k is the last germination time of the seeds, in days; n_i is the number of seeds germinated at time t_i (not the cumulative number, but that referred to the i-th observation), dimensionless; t_i is the time between the beginning of the experiment and the i-th observation, in days.

After this characterization, where part of the packages was opened, two different conditions were obtained: sealed and unsealed packages. These seed were stored in a cold room, for another three months in order to verify the effect of the conditions of packaging, as recommended by Nobre et al. [17]. The cold room were maintained with mean of 18°C of temperature and 60% of relative humidity, in the Laboratory of Seeds of the Faculty of Agronomy and Animal Science of the Federal University of Mato Grosso.

The experiment of seeds stored for 13 months (10 months in laboratory environment + 3 months in cold chamber) was arranged in a completely randomized design, in a factorial scheme with two moisture contents (9.82% and 7.84%), and two packaging conditions (sealed and unsealed), with four replications, totaling 16 plots. The data were submitted to the Shapiro-Wilk normality test [25], and to the analysis of variance, and the means were compared by the Tukey test at a 5% probability level. The analysis was performed through the computer program System for Analysis of Variance - SISVAR [26].

3. RESULTS AND DISCUSSION

The data from the amaranth BRS Alegria germination test were presented in an approximately normal distribution according to the Shapiro-Wilk normality test [25] and could be submitted to analysis of variance and to the mean test, except for the variables Moisture Content (MC) and MGT, which were made descriptive analyzes (Table 2).

It was verified that when the packages were sealed, there was a hygroscopic equilibrium between the seeds, with the final moisture content as the average of the initial moisture contents. When the packages were unsealed both lots absorbed moisture. In general, only the sealed 9.82% seed lot did not absorb moisture during storage (Table 3).

The increased moisture content for unsealed packages was expected for experimental conditions, since the relative humidity of 60% of the cold chamber influenced such observed results [15]. This shows that the packaging sealing kept the moisture content of the seeds lower than the unsealed packages for the two moisture contents studied, because when the packages are violated, their moisture content varies according to the variations of the humidity of the air, but when sealed, the polyethylene packages, which are semipermeable, create resistance to this exchange, keeping the initial moisture content for more time [16]. This also depends on the initial moisture content of the seeds, since in 7.84%, the seed also absorbed moisture, although a small amount.

Alves and Lin [15] found similar results in the variations of the initial moisture contents of 11% and 15% of bean seeds stored for 21 months for polyethylene packaging. These seeds enter into hygroscopic equilibrium with the environment, remaining with values between their initial moisture contents. For them, the polyethylene packaging was the one that best maintained the moisture content of the seeds.

For GP (Table 4), NGS (Table 6) and GSI (Table 7), the interaction between packing condition and moisture contents was statistically significant. For FGC (Table 5) only the packaging condition presented a statistical difference (Tukey, 0.5%).

In Table 4 is showed the mean values of germination percentage (PG) for amaranth BRS Alegria seeds. For the packaging conditions, it was observed that in the sealed packages, the lot with moisture content of 9.82% was higher germination than that of 7.84%. In unsealed packages, there was no statistical difference in the germination of the different of moisture contents. For the initial moisture contents, it was observed that the packaging sealing presented better germination results than the unsealed, mainly for the of initial moisture content of 9.82%.

The fact that the seeds with 9.82% initially presented a higher percentage of germination in relation to the 7.84% may have influenced the difference between the final moisture during refrigerated storage for 3 months. Most likely, the longer drying time (lot 7.84%) may have impaired the seeds and therefore this seed lot obtained a lower germination percentage at the end of the 10-month storage period under stressful ambient conditions. Its situation worsens after cold storage, decreasing more than 10 percentage points of its previous germination.

Similar results were found by Afonso Júnior and Corrêa [27], evaluating the drying effects of bean seeds (*Phaseolus vulgaris* L.). They observed that the decrease in germination is related to the increase of drying air temperature, drying time and storage time. On the other hand, the results obtained disagree with those authors who observed a decrease in germination in seeds with higher initial moisture content, probably because the humidity of 9.82% is already low humidity for these seeds [17,27].

Another possible cause for loss of germination in 7.84% seed lot was explained by Lin [28,29], who reported that the moisture absorption by the seeds to reach their hygroscopic equilibrium, causes the deterioration of the plasma membrane of seeds and decreases their physiological quality. This is also true for the unsealed 9.82% seed lot.

Table 2. Shapiro-Wilk normality test values for amaranth BRS Alegria seed germination test data

Test	n	Variables					
		MC	PG	FGC	NGS	GSI	MGT
Wc	16	0.776	0.955	0.975	0.955	0.975	0.863
Pr<Wc	16	0.001	0.585	0.913	0.585	0.916	0.021
Wt	16	0.887					

Wc: calculated Shapiro-Wilk value; Wt: tabulated Shapiro-Wilk value, according to number of plots (n) and significance level (5%); MC: moisture content; PG: percentage of germination; FGC: first germination count; NGS: non-germinated seeds; GSI: germination speed index; and MGT: mean germination time

Table 3. Variation of the initial moisture content, in %, of amaranth BRS Alegria seeds

Sealing of packaging	Moisture contente		Mean
	9.82%	7.84%	
Sealed	8.58	8.31	8.45
Unsealed	11.07	9.02	10.05
Mean	9.83	8.67	

Table 4. Germination percentage (GP), in %, of amaranth BRS Alegria seeds (seventh day)

Sealing of packaging	Moisture content		Mean
	9.82%	7.84%	
Sealed	77.50 Aa	51.50 Bb	64.50
Unsealed	35.00 Bb	47.00 Bb	41.00
Mean	56.25	49.25	

Means followed by the same letter, uppercase in line and lowercase in the column, do not differ statistically from each other by the Tukey test at a 5% probability level

Table 5. First germination count (FGC), in %, of the germination test of amaranth BRS Alegria seeds (third day)

Sealing of packaging	Moisture content		Mean
	9.82%	7.84%	
Sealed	71.50	51.00	61.25 a
Unsealed	33.50	47.00	40.25 b
Mean	52.50	49.00	

Means followed by the same letter do not differ statistically from each other by the Tukey test at a 5% probability level

Table 6. Non-germinated seeds (NGS), in%, of the germination test of amaranth BRS Alegria seeds (seventh day)

Sealing of packaging	Moisture contente		Mean
	9.82%	7.84%	
Sealed	22.50 Bb	48.50 Aa	35.50
Unsealed	65.00 Aa	53.00 Aa	59.00
Mean	43.75	50.75	

Means followed by the same letter, uppercase in line and lowercase in the column, do not differ statistically from each other by the Tukey test at a 5% probability level

Still, only the germination of the 9.82% seed lot in sealed packaging was higher than the pre-test data and is the only one that is within the pattern of seed marketing [30].

Observing Table 5, it is seen that the results of the first germination count were similar to those obtained in germination percentage (Table 4), most notably for 7.84% seed lot. There was no

difference between moisture contents (p-value of 6.18%), but it was to the packaging conditions. Then, it is possible to say that the moisture contents were not able to present differences in seed vigor [22].

The best result was obtained for sealed packages, regardless of the moisture content. Values between 50% and 69% of first count were also observed by Nobre et al. [17], for amaranth BRS Alegria seeds.

Storage in different packaging conditions presented important results, since sealed packages contributed to the maintenance of the physiological quality of amaranth seeds, regardless of the moisture content of the seeds, and it is therefore advisable to seal them.

As expected, the quantity of non-germinated seeds (Table 6) was lower for the sealed 9.82% seed lot, with no difference for the other treatments.

Although not discriminated in this work, a large portion of hard seeds was observed, being the main reason for its non-germination. In addition, other reasons for non-germination were seed deterioration and germination abnormality.

Similar values were found by Nobre et al. [17], for BRS Alegria amaranth seeds stored at 50% relative humidity and 26°C temperature, with moisture content of 10%. They found 17% of hard seeds under these conditions.

With the germination speed index results (Table 7), it is possible to see that unsealed packages did not present a statist difference in relation to sealed packages in 7.84% seed lot, but had a worse result in 9.82%, and it is recommended to seal for this moisture.

Regarding the moisture content, the 9.82% seed lot was superior when sealed but for 7.84% there is no difference in the packages conditions.

It is still possible to observe that the 7.84% seed lot maintained the GSI of the pre-test, while the 9.82% had the GSI improved when the packages were kept sealed, but was impaired when the packages were violated.

Thus, it can be considered that the germination speed index presented good sensitivity in the differentiation of amaranth seed lots. This test is of current and practical use, since it can be performed in conjunction with the germination test [22].

According to the data presented in Table 8, the 9.82% seed lot took longer to germinate than the 7.84%, for both the packaging conditions. This is because the faster the moisture uptake by the seeds for germination, the greater its deterioration and the lower its physiological quality [29,30].

The initial stages of hydration involve considerable rearrangement of the cellular constituents so that the seed becomes sensitive to rapid soaking. In this situation there may be release of solutes and macromolecules abundantly, causing problems due to the rapid entry of moisture into the seeds mainly when they are very dry. Damage to dried seeds is severely attenuated when soaking is slow [31], reducing the number of abnormal seedlings.

Also, with the TMG data it is possible to notice why the first germination count has values close to the final percentage of germination. It is because the amaranth germinates relatively fast, between 1.52 and 2.23 days. Similar results were found by Costa and Dantas [32], who obtained emergence of up to three days after sowing, reinforcing the rapid germination of amaranth.

Table 7. Germination speed index (GSI) of germination test of amaranth BRS Alegria seeds

Sealing of packaging	Moisture content		Mean
	9.82%	7.84%	
Sealed	24.88 Aa	19.78 Ab	22.33
Unsealed	8.91 Bb	15.37 Ab	12.14
Mean	16.89	17.58	

Means followed by the same letter, uppercase in line and lowercase in the column, do not differ statistically from each other by the Tukey test at a 5% probability level

Table 8. Mean germination time (MGT), in days, of the germination test of amaranth BRS Alegria seeds

Sealing of packaging	Moisture content		Mean
	9.82%	7.84%	
Sealed	2.08	1.50	1.79
Unsealed	2.20	1.69	1.94
Mean	2.14	1.60	

According to the results obtained, it was possible to state that the good seed results were found in lots with a moisture content of 9.82%, and no further drying was required, which would take longer and higher energy costs. However, it is recommended to seal the packages for their maintenance in the physiological quality during the storage period.

In addition, it was possible to verify a good resistance of the amaranth seeds as they have undergone a 10 month period, which was subject to environmental conditions (high temperature and great variation in relative humidity). The three months later stored in a cold chamber (and relatively high humidity), showed their ability to tolerate different environmental conditions.

In view of the data obtained and also as reported by Alves and Lin [15], in this work it was also observed that the polyethylene packages, when violated, have characteristics of permeable packages, while when properly sealed, they maintain their original characteristics.

4. CONCLUSION

It was concluded that the moisture content of 9.82% is adequate for the storage of Amaranthus cruentus BRS Alegria, with no further drying required.

It is recommended to seal the packages for the storage of amaranth seeds of BRS Alegria, as it maintains its physiological quality for longer.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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