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Physiological potential of the black sesame (*Sesamum indicum*) seeds in reply to the storage conditions

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Abstract

It was aimed to evaluate the physiological quality of sesame seeds stored in packaging and environments for a year. The completely randomized design in a factorial $3 \times 2 \times 7$ with four replications was used. The treatments were constituted of three types of packaging (kraft bag, PET bottle and plastic bag), stored in two storage environments (natural condition of laboratory and freezer at - 4°C and 80% RH), during 360 days, with reviews by physiological quality before storage and the others in a 60-day cycle. The permeable kraft paper packs along with the freezer environment, is inefficient for the conservation of vigor and viability of black sesame seeds during storage, due to allowing the exchange of water vapor of the seed with the environment. The vigor of black sesame seeds, with the exception of the electrical conductivity test, decreased over the storage period, regardless of the packaging used. The semipermeable packs of plastic bag and waterproof pet bottle are the most indicated for packaging of black sesame during the storage period, regardless of the environment.

Keywords: Oilseed, Physiological quality, deterioration, viability, vigor.

Abbreviations: P_Packing, E_Environment, SP_storage period, RH_relative humidity, MAPA_Ministry of Agriculture and Food Supply, SGT_ standard of germination test, FC_first count of SGT, SL_seedling of length, EC_electrical conductivity, CV_ Coefficient of variation.

Introduction

Sesame is one of the oldest oilseeds cultivated in the world and is widely cultivated in tropical and subtropical areas (Ashri, 2010). The oil extracted from its seeds is regarded as the most important product of the plant, with mean values between 48 and 56% (Silva et al., 2011), with a high content of unsaturated fatty acids, especially 47% oleic acid and 41% linoleic acid (Noble et al., 2013.) being used as food, in industry and as medicine. Its seeds still present around 30% of protein (Onsaard, 2012), and that makes it a product of high nutritional value. The plant is highly drought tolerant, grows well in many soil types and climates, being suitable for different options of rotations and crop sequences and intercropping with annual crops (Pham et al., 2010), for the Northeast and Midwest regions of Brazil. The storage phase is considered one of the most important for obtaining seed lots with higher physiological quality, and is the method by which the viability of the seeds can be preserved and their vigor maintained at a reasonable level during the period between sowing and the harvest (Bonner, 2008). The ability of seeds to maintain their quality during storage is influenced by several factors, including the initial physiological quality of the seed, mother plant vigor, climatic conditions during maturation, mechanical damage, drying conditions, proper moisture content, relative humidity, storage temperature, action of microorganisms and insects, types of packaging and physiological storage duration (Toledo et al., 2009). At maturity, there is an immediate start of the storage period before the harvesting operation, called in-field storage (Baudet, 2003). However, because the seed has a hygroscopic characteristic, it shows a considerable variation in water content due to relative humidity, thus a low water content of the seed, associated with low temperature of the storage environment and lower relative humidity are key points for the maintenance of viability for a prolonged period (Marcos Filho, 2005). Maintaining the viability and seed vigor during storage also depends on the type of packaging employed. Seeds conditioned in packaging which enables the exchange of water vapor with the environment can absorb water under high relative humidity, easily decaying seeds (Marcos Filho, 2005). Baudet et al. (2003) and Bonner (2008) classify the packaging regarding the exchange of water vapor in: permeable, semipermeable and impermeable, depending on the exchange of moisture that may occur between the seeds and the environment in which they are maintained. The permeable and semi permeable packaging allow greater exchanges of the seed with the environment in relation to impermeable packaging that do not allow this exchange.

Oilseeds such as sesame seeds, when improperly packaged, deteriorate with the increase of acidity. Storage conditions are determinant to guarantee physiological seed quality and although their quality cannot be improved, good condition during this period will contribute to keep them viable for a longer time, slowing down the process of deterioration (Nakagawa and Carvalho, 2012). However, investigative researches about the behavior of sesame during storage are rare and inconclusive. Lima et al. (2014) investigating the physiological quality of sesame seeds cv. BRS Seda of cream integument found that the seeds remain viable for of up to twelve months, when stored in a cold and dry room environment and the refrigerator, regardless the type of packaging used, while in the natural environment, the seeds remain viable for up to six months of storage. In the freezer

Table 1. Summary of analysis of variance, Mean squares (MS), derived from average values of first count (FC) of germination, standard of germination test (SGT), electrical conductivity test (EC) and seedlings length test (SL) applied of sesame (*Sesamum indicum*) seeds for twelve month storage

Variation Source	Desman of free laws			MS	
	Degrees of freedom	FC	SGT	EC	SL
Storage period (SP)	6	850.04*	368.61*	11361.78*	1333.88*
Environment (E)	1	892.74*	615.66*	262.95 ^{ns}	32.11 ^{ns}
Packing (P)	2	380.91*	243.05*	19.79 ^{ns}	18.55 ^{ns}
SP x E	6	286.87*	212.86*	317.41*	56.23*
SP x P	12	42.97 ^{ns}	46.30 ^{ns}	198.09 ^{ns}	4.37 ^{ns}
ExP	2	282.36*	247.89*	1.61 ^{ns}	4.08 ^{ns}
SP x E x P	12	32.49 ^{ns}	48.55 ^{ns}	76.20 ^{ns}	3.99 ^{ns}
Residue	126	45.39 ^{ns}	49.04 ^{ns}	131.12 ^{ns}	8.90 ^{ns}
CV (%)	-	11.73	9.55	10.69	19.49

*Significant at 5% probability by F test. ns not significant.



Fig 1. Percentage of normal germination seedlings obtained by first counting test (FC) applied to sesame (*Sesamum indicum*) seeds stored in plastic bags, kraft paper and plastic bottles, subject to controlled and non-controlled conditions in freezer during 360 days of storage. * Significant at 5% probability by F-test.

environment, paper packaging is more appropriate for the packaging of sesame seeds for up to twelve months. In a work conducted in by Oyekale et al. (2012) to evaluate the effect of organic products like neem (Azadirachta indica) and pepper, in powder form, on the physiological quality of sesame seeds of cream integument stored in airtight container and uncontrolled environment for approximately five months, observed that the use of aforesaid natural products can be used aiming to maintain the quality of sesame seeds during storage, but only for short and medium terms. Researches addressing the subject concerning packaging of the sesame seeds are related to the use of cream-colored seeds in relation to black seeds, and as acknowledged by Queiroga et al. (2010) and Silva et al. (2011) these two types of materials differ in terms of nutritional composition, and possibly during packaging. Furthermore, studies on storage of sesame seeds are of great importance for technicians and producers, providing a greater basis about the correct procedures for preservation of seeds of superior quality. Investigations about conservation of sesame seeds, particularly the black type are rare, and the information available insufficient, requiring more detailed investigations that convey greater security to producers in the quest for profitability. Thus, this study aimed to evaluate the physiological quality of black sesame seeds, packaged in different containers and environments, over a twelve-month storage period.

Results

By the result of the analysis of variance it can be verified that the SP alone influenced all the tests evaluated. The E, P and the interaction between these two factors influenced only the FC and SGT tests. The paired interactions SP × E influenced all tests applied. The paired interactions $SP \times P$ and a triple $SP \times E \times P$ did not significantly modify the results of the applied tests (Table 1). It was found that the PET bottles packaging applied to the laboratory environment for 60 days from packaging showed uniform averages with minor variations, being minimum of 4.31% (wb) and a maximum of 4.65% (wb) (Tables 2 and 3). Regarding the freezer environment, it was observed a maximum and minimum of 4.15 and 4.55% (wb), respectively. A plastic bag packing kept in laboratory and freezer environments has resulted into small changes in humidity over time. So packaging with these settings do not present great oscillations in water content even being stored under the uncontrolled environ-

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Packing (P)	Storage period (days)						
	0	60	120	180	240	300	360
Plastic bottles	4.82	4.65	4.52	4.31	4.31	4.41	4.5
Plastic bags	4.82	5.18	4.41	4.12	4.95	5.66	5.72
Kraft paper	4.82	5.55	4.21	4.91	6.49	5.84	4.94

Table 2. Percentage of water content (%) applied to sesame (*Sesamum indicum*) seeds stored in plastic bags, kraft paper and plastic bottles, subjected to uncontrolled laboratory conditions for a 360-day storage period.



Fig 2. Percentage of normal germination seedlings at standard germination test (SGT) applied to sesame (*Sesamum indicum*) seeds stored in plastic bags, kraft paper and plastic bottles, subject to conditions non-controlled, natural and controlled in freezer during 360 days of storage. * Significant at 5% probability by F-test.

Table 3. Percentage of water content (%) applied to black sesame (*Sesamum indicum*) seeds stored in plastic bags, kraft paper and plastic bottles, subjected to freezer controlled conditions for a 360-day storage period.

Packing (P)	Storage period (days)							
	0	60	120	180	240	300	360	
Plastic bottles	4.82	4.41	4.41	4.17	4.15	4.54	4.55	
Plastic bags	4.82	4.76	4.91	4.64	4.91	5.46	5.47	
Kraft paper	4.82	7.98	7.09	7.02	6.76	7.52	6.31	



Fig 3. Mean values of seedling of length test (SL) obtained from sesame (*Sesamum indicum*) seeds stored in plastic bags, kraft paper and plastic bottles, subjected to uncontrolled conditions - natural and controlled - freezer during 360 days of storage. * Significant at 5% probability by F-test.

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		FC (%)			
Environment (E)	Packing				
	Plastic bottles	Plastic bags	Kraft paper		
Laboratory	72.43Aa	74.71Aa	72.79Aa		
Freezer	71.93Aa	70.21Aa	57.36Bb		
SGT (%)					
Environment (E)	Packing				
	Plastic bottles	Plastic bags	Kraft paper		
Laboratory	90.43Aa	93.00Aa	91.50Aa		
Freezer	91.36Aa	90.64Aa 82.93Bb			

Table 4. Mean percentage values of the first count (FC) and the standard germination test (SGT) applied to sesame (*Sesamum indicum*) seeds for interaction packing \times environments throughout 360 days of storage.

Values represented by the different lower case letters in the column and upper case letters in the lines, show significant differences (Tukey test, $p \le 0.05$).



Fig 4. Reading for the electrical conductivity test (EC) applied to sesame (*Sesamum indicum*) seeds stored in plastic bags, kraft paper, PET bottle, subjected to uncontrolled conditions - natural and controlled - freezer storage for 360 days. * Significant at 5% probability by F-test.

ment for a long period. In relation to permeable packing (kraft paper) applied to the laboratory environment, oscillations were observed for the percentage of water content from 4.21% (wb) to 6.49% (wb). The same was observed for the permeable container (kraft paper) in the freezer environment, however, with minor variations between the periods than in the laboratory environment and with higher contents, ranging from 6.31% (wb) to 7.98% (wb) after 60 days of storage.

First count test (FC) and the standard germination test (SGT)

It was found that for tests of FC and SGT test the average percentage of normal seedlings obtained in each packing (plastic bottles, plastic bags and kraft paper) did not differ statistically amongst each other when stored in laboratory during 360 days of evaluation. However, significant differences were observed in the mean percentage values of FC and SGT tests for kraft paper packaging in relation to remaining ones, when packed in freezer environment for one year of packaging (Table 4). The packaging of pet bottle and plastic bag had mean scores on vigor of approximately 72%, while the kraft paper, positively less than 65%. Regarding the feasibility, we have the same behavior mentioned above with average of approximately 90% for PET bottle packaging and plastic bag, and 86% for the kraft paper. The percentage of normal seedlings obtained by the FC decreased with the storage time regardless of the environments tested (Fig. 1). The laboratory environment has presented a linear decrease of the FC test, while the environment freezer, with a quadratic behavior, decreased to approximately 180 days of storage, and subsequently has resumed with a slight growth by the end of 360 days, and the environment freezer exceeded the laboratory environment with higher percentage of vigor. This oscillation vigor of seeds stored in the freezer environment can be attributed to exposure to high relative humidity, a factor that has a direct influence on seeds due to its hygroscopic features (Fig. 1). The percentage of normal germination seedlings by the SGT (Fig. 2) decreased during the storage period for the laboratory environment, with no significant differences in germination percentages for the environment freezer during the evaluation period.

Seedling length (SL)

There was a reduction on seed vigor with storage time as for the results of SL test (Fig. 3). There was a decreasing linear fit for both storage environments. The length of seedlings to the laboratory environment was initially higher than the freezer environment; however, from 300 days of storage both seedling lengths become equal.

Electrical conductivity test (EC)

The readings of EC test applied to the sesame seeds in a plastic bag, kraft paper and plastic bottle, subjected to

uncontrolled conditions - natural and controlled - freezer for 360 days of storage decreased in both environments from a certain point by the end of storage period (Fig. 4). The freezer environment showed higher readings than the laboratory environment from the $120_{\rm th}$ day of storage until the $360^{\rm t}_{\rm h}$ day. In general, the reading of electrical conductivity decreased with time of storage, what indicates that the electrolytes released by seed in the water for moisturizing decreased at the end of 360 days of storage.

Discussion

The preservation of physiological seed quality in certain environmental conditions of temperature and relative humidity is influenced by the type of packaging used (Villela and Peres, 2004). In this study, we examined the percentage of water content, FC, SGT, SL and EC tests applied to sesame seeds stored in plastic bags, kraft paper and plastic bottles, subjected to uncontrolled laboratory conditions for a 360-day storage period. The PET bottles packaging applied to laboratory environment and freezer environment show uniform averages with minor variations for water content. This behavior can be attributed to the fact that the impermeable packing (plastic bottle) is able to isolate the seed from the influence of the external surroundings (air), not allowing moisture exchange with the environment. The plastic bag packing is type of packaging allows partial exchange with the storage environment, forcing the seeds to seek hygroscopic balance with their respective environments. So packaging with these settings do not present great oscillations in water content even being stored under the uncontrolled environment for a long period. Similar results were found by Alves and Lin (2003), working with bean seeds. Permeable packaging (kraft paper) offer no resistance to the exchange of water vapor from seed with the environment, being subjected to wide fluctuations in relative humidity of the laboratory environment and with the high relative humidity in the freezer environment. For Marcos Filho (2005) most of the seeds tends to suffer varied in their degree of moisture during storage period under uncontrolled conditions, following the fluctuations in relative humidity. The behavior of packaging in relation to the environments in a study confirms data found (Table 2 and 3). Medeiros and Eira (2006) classify the packaging, as the permeability water vapor, in permeable, semipermeable and impermeable. For Peske et al. (2006), all packaging can have a direct influence in the final physiological quality of the harvested seed. However, significant differences were observed just in the mean percentage values of FC and SGT for kraft paper packaging in relation to remaining ones, when packed in freezer environment for one year of packaging (Table 4). There were also significant differences in the average percentage values the FC and SGT concerning kraft paper packaging alone in relation to freezer and laboratory environments through the whole storage period. The kraft paper packaging was less efficient when packed in freezer environment, both for vigor as well as for viability, throughout storage period. All these results can be explained from the data of Tables 2 and 3, since the water content in the packaging of kraft paper, in freezer environment, were the most increased in the studied parameters. Probably this occurred due to the acceleration of cellular metabolism of the seed and consequently for the process of deterioration over the time and reduction of vigor and viability. This hypothesis corroborates the observations of Marcos Filho (2005), who considers that the water content of seeds is directly associated with deterioration. The speed and intensity of deterioration

and the activity of microorganisms are proportional to increases in water. The laboratory environment has presented a linear decrease of the first germination test, while the environment freezer has presented decreased to approximately 180 days of storage, and subsequently has resumed with a slight growth by the end of 360 days, and the environment freezer exceeded the laboratory environment with higher percentage of vigor. This oscillation vigor of seeds stored in the freezer environment can be attributed to exposure to high relative humidity, a factor that has a direct influence on seeds due to its hygroscopic features (Fig. 1). For the percentage of normal germination seedlings by the SGT (Fig. 2), decreased during the storage period for the laboratory environment, with no significant differences in germination percentages for the environment freezer during the evaluation period. Probably the laboratory environment has not offered ideal atmospheric conditions for the longevity of black sesame seeds, on account of its susceptibility to fluctuations in temperature and humidity of air, especially regarding permeable and semipermeable packaging, helping to accelerate the deterioration process of seeds stored in this environment. This result is consistent with reports by Marcos Filho (2005), for which storage under improper conditions contributes to the reduction of seed quality. In addition, it corroborates the results obtained by Pereira et al. (2013) with seeds of Jatropha top, whose percentage of germination and vigor decreased after storage in uncontrolled environment (laboratory), especially when storage was in kraft bag, followed by woven polypropylene packaging and cardboard drum. Normative Instruction No. 60, issued on December 2009, from the Ministry of Agriculture and Food Supply -Brazil (MAPA, 2009) sets the minimum criteria for marketing of sesame seed germination at 70% and purity at 98%, therefore, the seeds remained viable in all tested packaging environments and over twelve days storage with average germination at approximately 89% (Fig. 2). These results demonstrate that the quality of black sesame seed can be maintained in storage for a longer time compared to lighter seeds (Azevedo et al., 2003; Oliveira and Costa, 2009), which can be attributed to the centesimal chemical composition set among different materials (Queiroga et al., 2010; Silva et al., 2011). For Bonner (2008), when seeds are stored in a cold environment, will probably acquire moisture because of high relative humidity of these locations. Thus, the use of impermeable packaging prevents the increase in moisture content and rate of deterioration, offering vigorous seeds for a longer period of time. There was a reduction on seed vigor with storage time as for the results of seedling length (Fig. 3), an opposing fact from the one verified by Oliveira and Costa (2009) and Azevedo et al. (2003), who stored sesame seeds for nine and six months and concluded that their physiological quality did not decreased with storage time. It is recognized that vigorous seeds originate seedlings with higher growth rate, due to having higher processing capacity and reserves from storage tissues and their greater incorporation by the embryonic axis (Carvalho and Nakagawa, 2012). In general, the reading of EC decreased with time of storage, what indicates that the electrolytes released by seed in the water for moisturizing decreased at the end of 360 days of storage. This behavior is consistent with the test methodology (Vieira and Krzyzanowski, 1999). However, this result is not conformable with the rest of vigor and viability tests carried out in this study (Fig. 4). Vieira and Krzyzanowski (1999) clarifies that low electrical conductivity values correspond to lower release of exudates and indicate a high physiological potential, i.e., lower disruption intensity in the cell membrane system. According to Marcos Filho (1995), degradation of membrane systems is the first stage of the deterioration process of seeds. Nonetheless, the electrical conductivity test was not suitable either for evaluation of the strength in soybean seeds (Vieira et al., 2001) and pea (Panobianco et al., 2007).

Materials and Methods

Experimental conditions

The work was developed in the Drying and Storing Laboratory for Vegetable Products of State University of Goiás, Anápolis unit, during the period from May 2012 to May 2013. Black cv. sesame seeds were used, produced at the Emater Experimental Station in Anápolis –GO, Brazil. From the original batch of seeds were withdrawn homogenized samples and divided into 36 portions of 200g (0.44lb), which were conditioned in their respective packaging and storage environments.

Statistic design and treatments

The completely randomized design in factorial scheme 3 x 2 x 7 was used with four replications. The treatments consisted of storage of black sesame seeds in different types of packaging classified by Baudet (2003) as for the permeability of: (E1) permeable multiwall kraft paper container with two sheets, sealed with clear tape; (E2) impermeable pack of 250 mL (8.79 oz) plastic bottle with a thickness of 0.126 mm (0.004 in), sealed with common melted paraffin wax; and (E3) semipermeable plastic bag packaging sealed with transparent tape. After packed seeds were stored under natural conditions in a non-controlled laboratory environment with temperature ranging from 20 to 34°C and a relative humidity 47-90% (A1) and in freezers with temperatures averaging 4 ± 2 °C and average relative humidity of $80 \pm 3\%$ (A2), for a period of 360 days coming to a total of seven reviews of physiological quality every 60 days (0, 2, 4, 6, 8, 10.12).

Treatment applications and seed materials

In experiment installation, before packaging the seeds with the purpose of verifying any prior conditions of the seeds, a representative sample was withdrawn from the original batch, and the physiological quality was evaluated, and the data subsequently used as a result for the zero (0) storage period. Alongside with the prior physiological evaluation the water content was determined according to the Rule for the Analysis of Seeds (RAS), the standard greenhouse method, where the seeds were submitted to drying at 105 \pm 3 °C for 24 h, with results expressed in percentage (MAPA, 2009). The determination of water content was repeated at each assessment time of the physiological seed quality in all storage conditions.

Tests evaluated

The evaluation of physiological seed quality coming from the different treatments aforementioned was performed by the following tests: SGT, FC, SL and EC. SGT test which evaluates viability, conducted with four replications where 50 seeds/replicants, placed in acrylic boxes (gerbox), containing germitest paper substrate previously moistened with distilled water equivalent to three times the weight of the paper. The gerboxes were conditioned in a germination chamber at 28 °C. The evaluation was made on the $6_{\rm th}$ day after the

performance of the test. The percentage of normal seedlings was logged in (MAPA, 2009). FC test was conducted joint with SGT, aiming to evaluate vigor, and the percentage of normal seedlings present were considered on the $3_{\rm th}$ day after the start of the test. With the objective of evaluating vigor, the total SL test (radicle + hypocotyl) was measured with four replicates of 10 seeds were distributed in a straight line in a longitudinal direction on the upper third of the germitest paper previously moistened with distilled water equivalent to three times its weight. Subsequently, they were conditioned into germinator with 45 °C inclination, and 28 °C. After four days, the measurment of the total length of each seedling was performed with a millimeter ruler (Nakagawa, 1999).

In addition to the vigor tests, an EC test was conducted, applied in procedures described by Vieira and Krzyzanowski et al. (1999), in which four repetitions with 50 seeds, previously weighted in precision scales of 0,01g (0,000022 lb) were placed in plastic cups of 200 mL containing 75 mL of deionized water for moisturizing, placed and kept into a germination chamber at a constant temperature of 28 ° C for 8 h. The reading for the electrical conductivity DIGIMED CD-21 was performed by a conductivity meter and the results expressed in μ S cm⁻¹ g⁻¹ of seeds.

Statistical analysis

The values of water content of the seeds were not subjected to statistical analysis. The data on physiological seed quality were subjected to analysis of variance and the values expressed in percentage (FC and SGT) underwent logarithmic transformation (arcsen $\sqrt{\frac{x}{100}}$) to meet the presuppositions of homogeneity of variance and normality of residuals, required for the application the F test. When relevant, the averages of the qualitative factor (packaging and environment) were compared by Tukey test at 5% probability, while the quantitative factors (storage time) was subjected to regression study. The statistical calculations were performed using the statistical program SISVAR 5.1 (Ferreira et al., 2011).

Conclusion

The permeable packaging (kraft paper) provided greater variation in water content of black sesame, compared to pet bottle and plastic bag. The permeable kraft paper packaging combined with the freezer environment, proved inefficient for conservation viability and vigor of black sesame seeds along storage period, when compared to other kinds of packing. The black sesame seeds remained suitable for commercialization in all environments and packs tested during 360 days of storage. The packaging, semipermeable plastic bag and impermeable pet bottle, are the most suitable for keeping black sesame along the storage period, regardless of environment. The vigor of black sesame seeds, except for the electrical conductivity decreases over the period of storage, independent of environment and tested packaging.

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