



Storability of landrace fava bean seeds in different packaging materials

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ABSTRACT: The present study evaluated, through physical and physiological tests, the seed quality of a landrace fava bean variety in different packaging materials and storage periods, aiming to guarantee the conservation of their quality in seed banks and facilities. The methodology used was the one recommended by the Rules for Seed Analysis (RAS - Brasil, 2009) and minimum descriptors for *Phaseolus lunatus* L. The data were subjected to analysis of variance by the F-test at 5% significance to verify the presence of significant interactions and the individual effects of each treatment. The test of multiple comparison of means was performed by the Tukey test at 5% significance between common treatments. The Dunnett test was used at 5% significance to compare the control means with the means of the treatments. Regression analysis was performed to study the effect of storage on the variables evaluated. All analyses were performed using the R software. From the results, it can be concluded that the storage of fava bean seeds is viable in small rural properties in impermeable packaging materials and aluminized polyethylene bags. The germination and vigor of the seeds analyzed in this study decreased throughout the storage period regardless of the type of packaging used. It is not recommended to store fava bean seeds in permeable paper packaging as they have the lowest quality indices (germination and vigor) at the end of the storage period.

Key words: *Phaseolus lunatus*, germination, vigor, packaging, conservation.

Armazenabilidade de sementes crioulas de feijão-fava em diferentes embalagens

RESUMO: O presente trabalho teve por objetivo avaliar por meio de testes físicos e fisiológicos a qualidade das sementes de uma variedade crioula de feijão-fava em diferentes embalagens. (1 -Embalagem cartonada mista composta por camadas de plástico, papel cartão e folha de alumínio impermeável- CN; 2- garrafa plástica semipermeável - PET; 3- Pote plástico rígido impermeável - PEAD; 4- pote de vidro transparente quebrável impermeável - VIDRO; 5- saco plástico flexível impermeável PEBD aluminizado; 6- saco de papel permeável) e períodos de armazenamento de (90, 180, 270 e 360 dias), visando garantir a conservação da qualidade delas em casas e bancos de sementes. A metodologia utilizada foi a preconizada pelas Regras de Análise de Sementes (RAS - Brasil, 2009) e por descritores mínimos para *Phaseolus lunatus* L. Os dados foram submetidos a análise de variância pelo teste F ao nível de 5 %, afim de verificar a presença de interação significativa e dos efeitos simples de cada tratamento. Foi realizado o teste de comparação múltipla das médias pelos testes *Tukey* ao nível de 5 % de significância entre os tratamentos comuns. Para comparar as médias da testemunha com os tratamentos comuns recorreu-se ao teste *Dunnett* ao nível de 5 % de significância. Para estudar o efeito do armazenamento sobre as variáveis avaliadas foi feita a análise de regressão. Todas as análises foram feitas com o auxílio do R por meio de programação. Pelos resultados, pode-se concluir que o armazenamento de sementes de feijão-fava: é viável em pequenas propriedades rurais em embalagem impermeável; saco de polietileno aluminizada. A germinação e o vigor das sementes analisadas diminuiram ao longo do período de armazenamento, independentemente do tipo de embalagem utilizada. Não é recomendado armazenar sementes de feijão-fava em embalagens permeáveis de papel por apresentarem os menores índices de qualidade (germinação e vigor) ao final do período de armazenamento.

Palavras-chave: *Phaseolus lunatus*, germinação, vigor, embalagem, conservação.

INTRODUCTION

Fava bean (*Phaseolus lunatus* L.), a plant species mainly grown by family farmers, is a vegetable of significant importance in both human and animal nutrition as it provides proteins and amino acids, increases the income, and ensures food sovereignty to farming families. In the scenario

involving climate change, fava beans show great rusticity and adaptability to the semi-arid climate. From this perspective, preserving seeds under ideal moisture and temperature conditions ensure the reduction of metabolic activities and promotes seed storability.

Adequate seed storage in appropriate packaging materials contributes to maintaining seed germination and vigor in seed banks and other storage

facilities. Seeds need to be adequately stored in order to ensure their physical and physiological quality and minimize future losses in production, otherwise, efforts to develop materials and crop management practices for production might be lost. It is common to find landrace fava bean seeds traditionally stored in PET bottles in rural family properties, which are kept throughout generations. The work carried out by ancestors, whose seed stocks are in family properties, has great genetic value for the biodiversity and food security of local populations and the entire Brazilian society (COPACHESKI et al., 2013).

Seed quality is influenced by storage conditions in the period between harvest and sowing. During storage, high temperature and moisture conditions are the main factors that influence seed quality, especially vigor. Relative moisture is related to the seed water content and controls the metabolism of different processes. Furthermore, temperature influences the speed of biochemical processes and indirectly impacts the seed water content (CARVALHO & NAKAGAWA, 2012). The type of packaging used and the environment where the seeds are stored are important since deterioration is also associated with the characteristics of the package that contains the seeds, determining the amount of water vapor exchange between seeds and the atmospheric and environmental conditions under which seeds are stored. In this scenario, one of the strategies to conserve genetic resources involves the creation of Germplasm Banks, which routinely carry out activities of morphological, geographic, and molecular characterization of accessions (HILL et al., 2013; MACIEL et al., 2016; CARVALHO et al., 2017). Another important strategy is *in situ* and *on farm* conservation, which occurs in production units and has been performed by farmers for millennia. These practices are based on a continuous process of evolution and adaptation, in which new variants appear and are challenged by natural and artificial selection (STELLA, 2006; EMBRAPA, 2010). In this scenario, given the need to conserve biodiversity and the genetic potential of this material to maintain its viability for a long period, it is necessary and indispensable to provide effective storage conditions in order to promote efficient and sustainable technologies for the conservation of the quality, viability, vigor, and germination of landrace fava bean seeds during storage. Therefore, this study evaluated the physical and physiological quality of landrace fava bean seeds during storage at ambient temperature to identify the best packaging and storage conditions to favor the conservation of seed quality in seed banks and storage facilities.

MATERIALS AND METHODS

Landrace fava bean seeds of the Lagartixa variety harvested 15 days before the experiment was set up were used in the experiment. The material came from a planting area located in the Santa Ingracia settlement in the municipality of Bocaiuva – MG. The seeds were selected by separating malformed, hollow, and seeds with impurities (inert material and other seeds), generating a material with 100% purity. The experimental design was completely randomized (DIC), with a $6 \times 4 + 1$ factorial arrangement corresponding to six treatments (1- mixed carton pack consisting of plastic, paperboard, and waterproof aluminum foil layers – CN; 2- semi-permeable plastic bottle - PET; 3- impermeable rigid plastic pot – PEAD; 4- impermeable transparent breakable glass pot – VIDRO; 5- waterproof aluminized flexible plastic bag - PEBD; 6 - paper bag (permeable)) and four storage periods (90, 180, 270, and 360 days), with an additional or control treatment (0 days of storage) and four replications of 25 seeds per treatment. Each pack consisted of 118 g of seeds stored for 360 days, starting on September 28, 2019, and ending on September 28, 2020. The seeds were stored in hermetically closed packs with impermeable plastic film, except for the paper bag, which was filled with adhesive tape. All packs used in the experiment were reused, except for the single-use paper bags purchased from the local market. The packs were cleaned with a 10% sodium hypochlorite solution, in which they were immersed for 10 minutes, and then washed with neutral detergent and running water, after which they were dried for 24 hours at ambient temperature. The temperature and moisture conditions of the storage location were monitored using an EXTECH thermo-hygrometer. Storage was carried out under ambient conditions in a room located in Block C of ICA at UFMG for 360 days, and the tests were evaluated every 90 days. The physical quality of the fava bean seeds was evaluated for the water content, 100-seed weight, seed biometry (length, width, thickness, size, and shape), seed color, and sieve retention. The seed water content was determined according to the methodology established in the Rules for Seed Analysis (BRASIL, 2009) using the drying oven method at 105 ± 3 °C for 24 hours, with results expressed in % of water content (w.b.). The 100-seed weight was determined using eight subsamples of 100 seeds from the pure seed portion. The seeds were counted manually and then weighed in an analytical balance accurate to 0.0001 g (BRASIL, 2009). The mean weight result was expressed in grams. Seed biometry was obtained with the following data:

length (longitudinal), width (transversal), and thickness, measured using a digital caliper (accurate to 0.01 mm) and employing four replications of 25 seeds per treatment. The tegument color of the fava bean seeds of the Lagartixa variety was described according to the classification chart of MUNSELL (1975). The evaluation of physiological quality was performed with germination and vigor tests (first germination count, germination speed index, and fresh and dry mass of roots and shoots). Four replications of 25 seeds were used per treatment for the germination test. The seeds were sown in germitest paper moistened with distilled water at a proportion of three times the paper weight. Next, the seeds were transferred to BOD incubators and kept at 30 °C under constant light. The evaluations were performed on the fifth and ninth days after the test was set up, and the results were expressed in percentage of normal, abnormal, dead, and hard seeds (BRASIL, 2009). Along with the germination test, the first count test and the germination speed index were determined with evaluations performed during five days by daily counting the number of seedlings that showed radicular protrusion. The results of this test were obtained by the number of normal seedlings, determined by occasion of the first count, i.e., the germination speed index was determined on the fifth day after the test was set up by employing the equation proposed by MAGUIRE (1962), whose results are non-dimensional:

Equation 2:

$IVG = (G1/N1) + (G2/N2) + \dots + (Gn/Nn)$, where:

IVG: germination speed index;

G: number of seedlings germinated on the day;

N: number of days after sowing.

The fresh weight was obtained after nine days. The emerged seedlings were sectioned, and the shoot and cotyledons were separated from the roots. These structures were placed in paper bags and weighed in a precision balance accurate to 0.0001g to obtain the fresh matter. Next, the material was dried to constant weight in a forced-air oven at 65 °C. After this period, the material was weighed in a precision balance accurate to 0.001g to obtain the dry matter according to the methodology proposed by CAVALCANTE et al. (2012). The data were subjected to analysis of variance by the F-test at 5% probability to verify the presence of a significant interaction and the individual effects of each treatment. Next, the test of multiple comparison of means was evaluated by Tukey's test at 5% probability. The comparison of means of the control with the other treatments was performed by Dunnett's test at 5% significance. Regression analysis

was performed to evaluate the effect of storage on the variables analyzed. The analyses were performed using the software R.

RESULTS AND DISCUSSION

Fava beans are a species that shows high variability in size, tegument color, and identification characteristics, which are essential for the knowledge of the species. Identified by the MUNSELL chart (1975), the Lagartixa variety shows a gray-pink coloration with dark black spots covering 50% of the seed, a halo of the same color as the tegument, bicolor and uniform seeds, intermediate brightness, and presence of venations on the testa. The fava bean seeds used in the present study had dimensions ranging from 12.88 to 14.68 mm in length, 7.77 to 8.79 mm in width, and 5.59 to 6.90 mm in thickness. The mean weight ranged from 45.44 to 56.90 g/100 seeds (Table 1) in studies conducted by SILVA et al. (2010), and the sizes range from very small (lower than 20 g/100 seeds) to large (more than 40 g/100 seeds), with the seeds of the fava bean variety Lagartixa used in the present study being classified as large. When evaluating the days of storage, the seeds stored in PET bottles after 270 days showed a significant difference compared to the mixed carton packaging and differed from the other materials by the Tukey test at 5% significance. In turn, the control differed significantly from the PET packaging at 5% by the Dunnett test with 270 days of storage. There was a significant interaction (Table 2) between the levels of factor A (storage time) and factor B (type of packaging) and for the contrast between the control and the other treatments, except for moisture (%), indicating the difference between the mean of the control treatment and the mean of the values obtained for the factorial treatments. The average temperature during storage ranged from 17 °C to 28,1 °C, with the minimum temperature occurring in August 2019 and the maximum in September 2018. Relative air humidity during the experimental period ranged from 30 to 88 %, with the maximum value in December 2018 and the minimum in August 2019 (Figure 1). The months of October and December 2018 and August 2019 showed relative air humidity means above 65% (Figure 1), favoring fungal proliferation and accelerating the metabolism and deterioration of stored seeds. Overall, fungi reduce seed quality due to the heating caused by respiration and the production of harmful enzymes and toxins. Among the enzymes produced, the most important are celluloses, pectinases, amylases, lipases, proteases, and nucleases (HALLOIN, 1986), responsible for altering the chemical and structural

Table 1 - 100-seed weight (g) of landrace fava bean seeds during storage in different packaging materials (Montes Claros, MG, 2020).

Type of Packaging	Time of storage (days)			
	90	180	270	360
1 - CN	50.02 a	46.95 a	56.90 ab	43.96 a
2 - PET	46.76 a	46.54 a	62.74 a*	47.35 a
3 - PEAD	48.17 a	45.28 a	46.06 c	46.24 a
4 - GLASS	47.56 a	46.95 a	45.88 c	43.37 a
5 - PEBD	47.86 a	47.76 a	48.40 bc	45.48 a
6 - PAPER	48.15 a	45.45 a	45.14 c	45.97 a
Control	46.93			

Means followed by the same letter in the column do not differ by the Tukey test at 5% significance. Means followed by * differ from the control by the Dunnett test at 5 % significance.

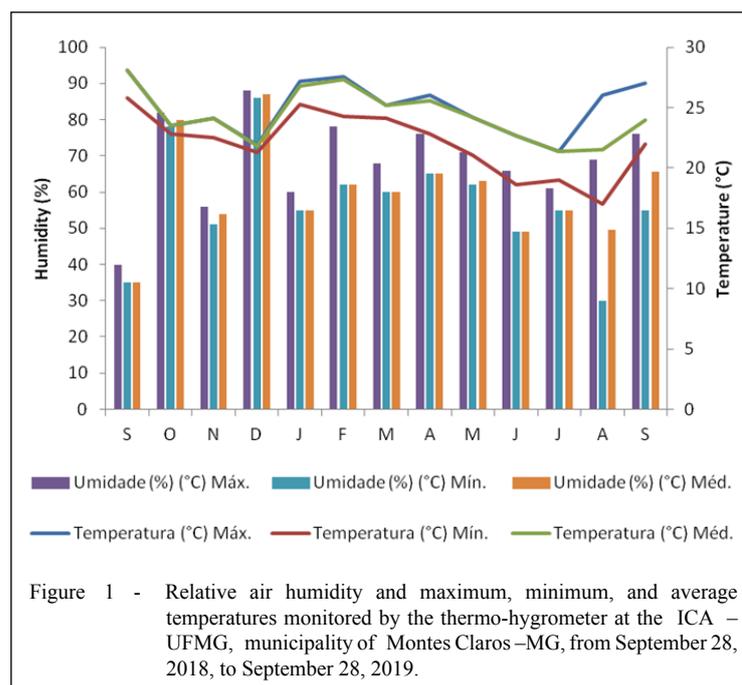
composition of seeds, leading to the depletion of nutrient reserves, increasing the concentration of free fatty acids, and causing damage to proteins and nucleic acids. Toxins, in turn, directly or indirectly affect the integrity of cell membranes by intensifying the exudation of solutes and causing tissue discoloration and necrosis. The fava bean seeds were stored with an initial water content of 10.69% (Table 3). According to COIMBRA (2007), the initial seed water content is an important parameter for the standardization of physiological quality assessments, highlighting that high water contents can favor seed performance in assays. The paper bag packaging showed the highest water content after 270 days of storage, differing from the other materials and the control by the Tukey and Dunnett tests at 5% significance (Table 3). However, after 360 days, the paper bag packaging showed one of the lowest water contents. According to COPELAND & MCDONALD (1995), the hydration and drying cycles in seeds reduce their physiological quality. The results indicated that the mean water content in fava bean seeds stored in paper bags range from 3.06 to 7.68 in the period from 90 to 270 days of storage

(Table 3). Graph 2 shows that the seeds stored in paper bags reached the highest water contents, showing higher variation in relation to the control and other packing materials after 360 days of storage and, consequently, showing the highest deterioration. From 270 days until the end of storage, the seeds in paper bags lost water when achieving hygroscopic balance with relative air humidity. Furthermore, according to table 3, the glass pot also differed statistically from the control by showing a high water content in stored seeds, which was possibly caused by sealing problems in the glass pot used for storage. The mixed carton (CM), PET bottle, and aluminized plastic bag (PEBD) packaging materials did not differ statistically, showing little variation in the water content during the entire seed storage period. However, these materials differed statistically from the others at 5% significance by the Tukey test, and kept their water contents close to the control, not differing from it by the Dunnett at 5% probability. The mixed carton and plastic pot materials did not differ regarding the moisture contents compared to the control during the entire evaluation period, maintaining both the metabolism and insect activity

Table 2 - Mean squares referring to the presence of significant interactions and individual effects by the F-test in the ANOVA.

FV	QM							
	First count (%)	Normal seedlings (%)	Moisture (%)	Fresh shoot matter (g)	Fresh root matter (g)	Dry shoot matter (g)	Dry root matter (g)	100-seed weight (g)
Factor A	15539.78**	14404.44**	22.35*	1768.59**	130.59**	65.91**	1.14**	134.95**
Factor B	1441.47**	1135.60**	27.15**	165.54**	4.87**	7.23**	0.0**	64.34*
AxB	572.04**	572.04**	10.89**	69.19**	3.53**	3.36**	0.04**	54.73**
Test_Vs_Control	3028.51**	2595.84**	1.012 ^{ns}	121.89**	32.25**	34.32**	0.43**	2.31 ^{ns}
Residual	119.31	80.37	0.39	12.09	0.52	0.66	0.01	23.12

** not significant at 1 %, 5 %, * not significant by the F-test.



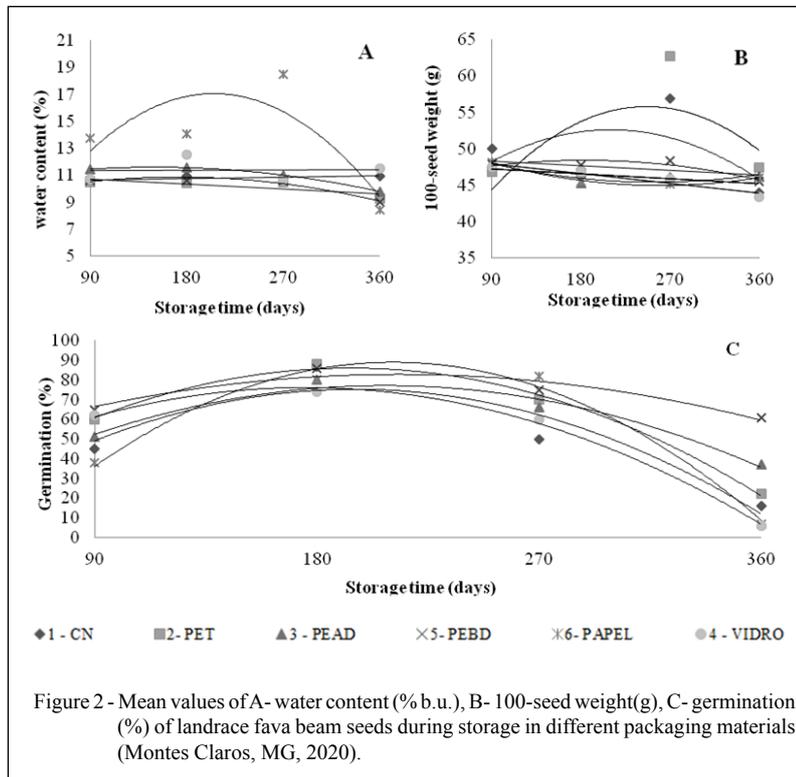
considerably reduced. According to HARRINGTON (1972), some general precepts regarding the maintenance of viability throughout storage can be applied when considering water contents from 5% to 14% and storage temperatures from 0 °C to 50 °C: each 1% reduction in the seed content doubles the viability period; each 5.6 °C decrease in the storage temperature doubles the viability period. Water contents above 40% favor germination. In turn, water contents from 18% to 30% trigger seed deterioration processes, whereas seeds stored with water contents from 18% to 20% tend to show intense respiratory activity, which, on the other hand, generates heat and

potentiates deterioration. Conversely, below 20% moisture, the metabolism and insect activity are considerably reduced, and seeds stored with water contents below 4% to 5% are immune to insect and fungal attacks during storage (BEWLEY & BLACK, 1994). The paper bags maintained the water content of stored seeds above 13% from 90 to 270 days of storage, reaching 18.48% after 270 days (Figure 2A), which allows the intensification of deterioration and the activity of insects and fungi. Insect activity can be reduced with the reduction in temperature and relative moisture in the storage environment since it is intensified in environments with

Table 3 - Water content (% b.u.) of landrace fava bean seeds during storage in different packaging materials (Montes Claros, MG, 2020).

Type of Packaging	Time of storage (Dias)			
	90	180	270	360
1 - CN	10.57 b	10.97 c	10.77 b	10.93 ab
2 - PET	10.49 b	10.42 c	10.49 b	9.26 cd *
3 - PEAD	11.45b	11.57 bc	11.00 b	9.80 bc
4 - GLASS	10.77 b	12.54 b*	10.68 b	11.49 a
5 - PEBD	10.62 b	10.63 c	10.62 b	9.00 cd*
6 - PAPER	13.74 a *	14.06 a*	18.48 a*	8.48 d*
Control	-----10.69-----			

Means followed by the same letter in the column do not differ by the Tukey test at 5% significance. Means followed by * differ from the control by the Dunnett test at 5 % significance.



temperatures higher than 25 °C and when the seed water content surpasses 13 % (MARCOS FILHO, 2005). Impermeable materials prevent increases in the seed water content, whereas permeable materials undergo great variations in the water content, achieving hygroscopic balance with the moisture of the storage environment. In Graph 2B, the PET bottle packs showed higher mass and 100-seed weight compared to the other materials. The lowest 100-seed weight was achieved by seeds stored in

paper bags, followed by glass pots, with these two materials showing weight variations throughout storage. The maintenance of high dry matter levels depends directly on the environment since unfavorable temperature or moisture conditions and the action of microorganisms contribute to accelerating the respiratory process and the oxidation of reserve substances, with reductions in seed weight (MARCOS-FILHO, 2015). The germination percentage, as shown in table 4, did not follow the expected reduction

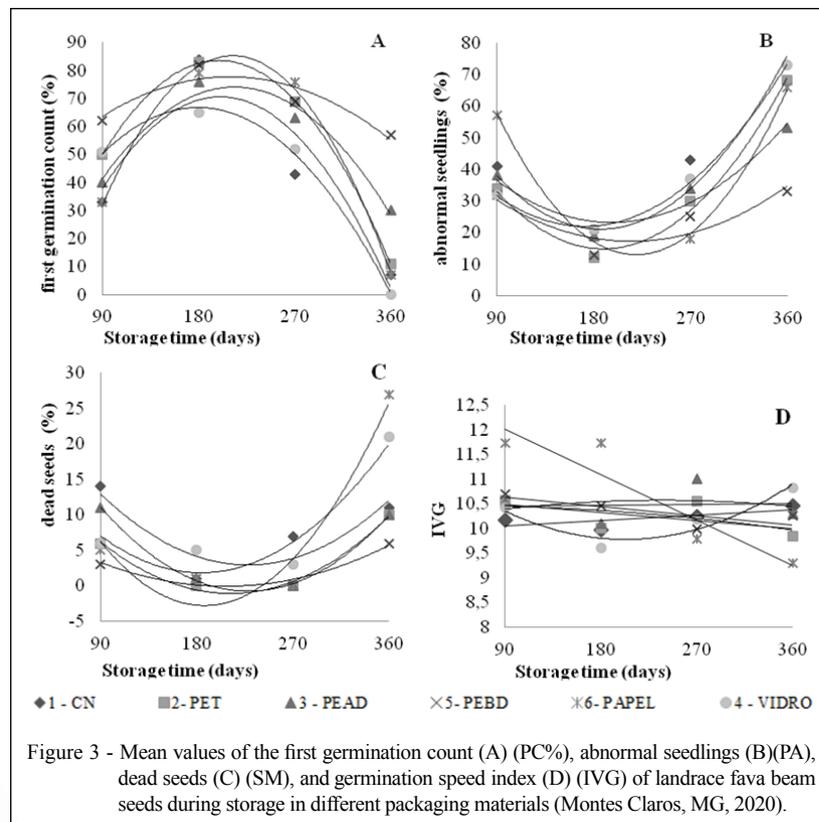
Table 4 - Germination (%) of landrace fava beam seeds during storage in different packaging materials (Montes Claros, MG, 2020).

Type of Packaging	Time of storage (days)			
	90	180	270	360
1 - CN	45.00 bc*	87.00 a	50.00 c*	16.00 c*
2 - PET	60.00 ab*	88.00 a	70.00 ab	22.00 bc*
3 - PEAD	51.00 abc*	80.00 a	66.00 abc	37.00 b*
4 - VIDRO	62.00 ab*	74.00 a	60.00 bc*	6.00 c*
5 - PEBD	65.00 a	86.00 a	75.00 ab	61.00 a*
6 - PAPEL	38.00 c*	80.00 a	82.00 a	7.00 c*
Control	83.00			

Means followed by the same letter in the column do not differ by the Tukey test at 5% significance. Means followed by * differ from the control by the Dunnett test at 5% significance.

pattern over the storage time since germination increased again after 180 days. The high temperatures and moisture after 90 days may have compromised germination, showing the lowest values during this period. Similar results were found by AZEVEDO et al. (2003a) when studying the influence of packing materials and storage conditions on the vigor of sesame seeds, observing that, in plastic and paper bags, the decrease in vigor oscillated between the fourth and sixth month, which could be explained by the temperature and humidity increase during these months, significantly influencing seeds due to their hygroscopicity. Table 4 shows that most packing materials used in the present study did not maintain a minimum germination potential for commercialization purposes, and only the seeds stored in aluminized plastic bags (PEBD) were marketable as per the classification of common beans. According to current regulations, fava bean seeds are not classified for commercialization. However, this classification is enabled when using common bean categories (*Phaseolus vulgaris* L.), in which the minimum germination is 70% for basic seeds and 80% for certified (C1 and C2) or non-certified seeds (S1 and S2) of first and second

generation (BRASIL, 2009). COELHO et al. (2011) studied landrace seeds of different bean cultivars, which showed high physiological potential in relation to the commercial cultivar as a function of the higher initial germination percentage and field emergence. The values of the present study, with fava bean cultivars, were lower than those found by COELHO et al. (2011). The results of the germination test highlighted that the seeds stored in PEBD and PET packs showed higher means of normal seedlings, with 70 and 60% germination during the storage period, respectively, and differing from each other and from the other materials, with values equal to or higher than 1.5 % and reaching 21.5% germination, as seen in table 4 and figure 2C. In the first germination count, the highest percentages of normal seedlings were also obtained for PEBD and PET materials, thus also highlighting the higher vigor. These two materials differ regarding vigor, with the PEBD material performing better than PET bottles (Figure 3 A). For dead seedlings, the paper bags and glass pots showed the highest mean values and differed from the others, but not from each other (Figure 3 C). The hard seed variable was not influenced ($P \leq 0.05$) by the type of packaging; therefore, the results

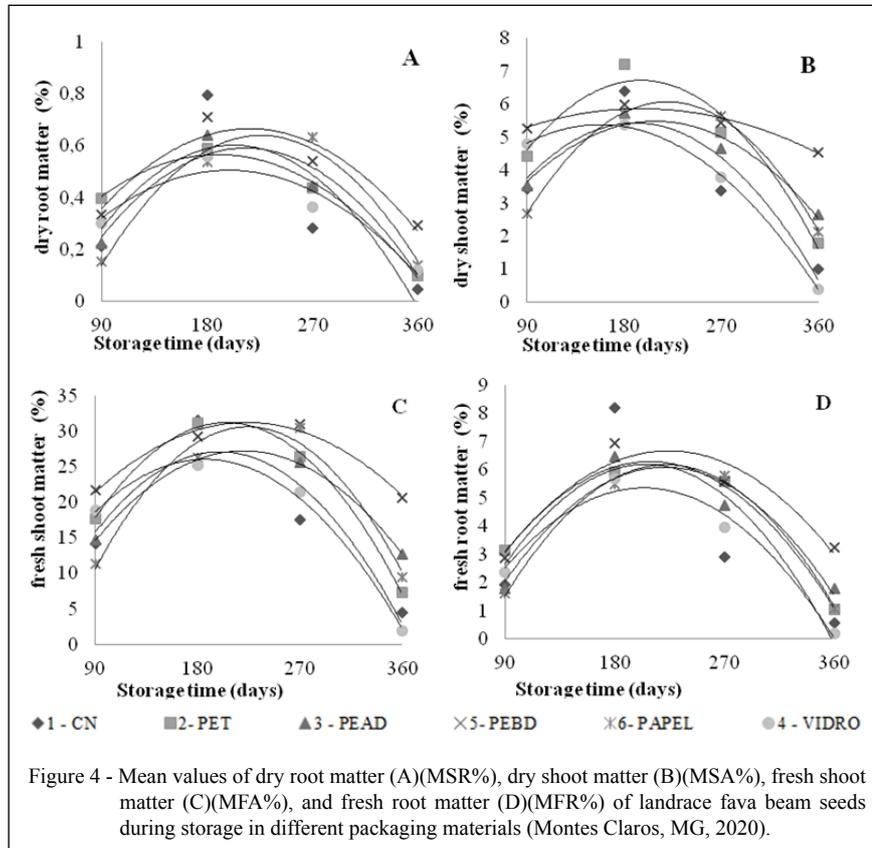


were not shown. Graph 3D shows the mean results of the germination speed index (IVG). There was an effect ($P \leq 0.05$) of the treatments on the IVG, separated into three different groups, with the paper bags showing the lowest IVG, decreasing gradually during storage, which again highlights the low potential of seeds stored in this type of packaging, followed by PEAD and glass materials, which showed IVG variations. In the CN pack, the IVG almost did not vary, remaining constant during the storage period. The PEBD and PET materials showed IVG reductions but little variation, with the highest IVG mean achieved by the PEBD material, which was close to the control, followed by PET, which is another use option for farmers (Figure 3 D). The seed germination capacity is not necessarily associated with the formation of vigorous plants since physically damaged seeds can still germinate, although with less vigor. Thus, seeds with similar emergence rates can have different germination speed indices (MARCOS FILHO, 2015). The highest values of primary root weight in the seedlings were obtained when using PEBD and PET materials, whereas the lowest values were observed for seeds stored in paper bags (Figure 4). The PEBD material gave origin to

seedlings with higher shoot and root weight, whereas seeds stored in glass pots resulted in seedlings with lower root and shoot fresh mass values. The highest root and shoot dry mass contents (Figure 4) occurred in the PEBD packs, whereas the lowest values were obtained by the seeds stored in glass pots. The PEBD material showed the highest mean values for the fresh and dry matter data, equaling the control and differing from the other materials (Figure 4). The packing materials influenced plant development since the fresh and dry matter values differed significantly between treatments, with the best preservation of the physiological potential occurring in the PEBD packs, followed by PET bottles, maintaining higher germination and vigor. Seed physiological quality is extremely important to evaluate a seed lot as it directly influences seed performance, favoring a higher speed in metabolic processes and the faster and more uniform emergence of primary roots during germination (MINUZZI et al., 2010).

CONCLUSION

The germination and vigor of the seeds analyzed in this study decreased over the storage



time regardless of the type of packaging used. The PEBD and PET materials minimized changes in the water content, weight, dry matter, germination, and vigor of landrace fava bean seeds during storage at ambient conditions compared to permeable paper bags. However, the impermeable PEBD bags (aluminized polyethylene bag) were promising for seed storability. It is not recommended to store fava bean seeds in permeable paper bags.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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