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The effect of fruit maturity on the physiological quality and conservation of *Jatropha curcas* seeds[1]

Qualidade fisiológica e conservação de sementes de *Jatropha curcas* em função da maturidade dos frutos

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ABSTRACT - The aim of this study was to evaluate the effect of fruit maturation stage on the physiological quality of *J. curcas* seeds during storage. Thus, seeds were extracted from fruits harvested at different maturity stages based on external color, i.e., yellow, yellow-brown and brown (dry fruits). After natural drying, the seeds were packed in Kraft paper bag and stored for 18 months at laboratory environment. Initially and every three months, the seeds were evaluated for moisture content, germination, first count of germination, accelerated aging, cold test, electrical conductivity and emergence. There was reduction in seed physiological quality, with decrease in germination and vigor, especially after nine months of storage. The seeds extracted from yellow and yellow-brown fruits are the most vigorous and can be stored for up to nine months without loss of physiological quality.

Key words: Germination. Vigor. Storage. Seed deterioration.

RESUMO - O objetivo desse trabalho foi avaliar o efeito do estádio de maturação dos frutos na qualidade fisiológica das sementes de *J. curcas* L. durante o armazenamento. Assim, sementes foram extraídas de frutos colhidos em três estádios de maturação com base na coloração externa da casca, ou seja, amarelo, amarelo-marrom e marrom (frutos secos). Após secagem natural, as sementes foram armazenadas por 18 meses em sacos de papel Kraft, em ambiente de laboratório. Inicialmente e a cada três meses, as sementes foram avaliadas quanto ao grau de umidade, germinação, primeira contagem de germinação, envelhecimento acelerado, teste de frio, condutividade elétrica e emergência. Houve redução da qualidade fisiológica das sementes durante o armazenamento, com queda na germinação e no vigor, principalmente a partir de nove meses de armazenamento. As sementes extraídas de frutos com coloração externa da casca amarela e amarelo-marrom são mais vigorosas e podem ser armazenadas por até nove meses sem perda da qualidade fisiológica.

Palavras-chave: Germinação. Vigor. Armazenamento. Deterioração de sementes.

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INTRODUCTION

Jatropha curcas L. is a promising source of raw material for biodiesel production and its seeds have high oil content (around 38%) (DIAS, 2011; TIWARI; KUMAR; RAHEMAN, 2007). In addition, greenhouse gas emissions from internal combustion engines can be reduced by substituting fossil fuels for the oil obtained from seeds of this species (JONGSCHAAP *et al*., 2007; OPENSHAW, 2000).

J. curcas seeds are of high quality upon reaching physiological maturity (SILVA *et al*., 2011). However, considerable lack of uniformity in fruit maturation makes it difficult to monitor this process in the species (SILIP *et al*., 2010) and the outside color of the fruit has been used as a parameter for determining when to harvest the seeds.

The fruit is generally harvested when the peel is yellow (KAUSHIK, 2003; PESSOA *et al*., 2012; SILVA *et al*., 2012; SOWMYA *et al*., 2012) or brownish-yellow (SILVA *et al*., 2012). Seeds of high physiological quality are best obtained in these two stages.

After harvest, the seeds can be sown or otherwise stored for sowing at a later date. The physiological quality of the seeds soon after harvest is one of the factors that affects their longevity and storage potential. According to Morais (2008), when *J. curcas* seeds are stored with good initial quality (94% germination), they are viable up to eight months; however, storage conditions also affect their conservation. Pereira *et al*. (2013) recommend that *J. curcas* seeds be placed in cold storage (5 ºC) in Kraft paper or woven polypropylene bags. Another option is a cardboard drum in an environment without temperature and moisture control. According to Oliveira (2013), seeds remain viable and with acceptable levels of vigor for at least 12 months when stored in a climate-controlled environment, such as a cool storage room (20 ºC) or cold storage (10 ºC), in suitable packaging (plastic bags).

Although some studies have defined the best conditions for storage of *J. curcas* seeds (GUZMAN; AQUINO, 2009; OLIVEIRA, 2013; PEREIRA *et al*., 2013; PINTO JÚNIOR *et al*., 2012; WORANG *et al*., 2008), there is little information on the relationship between the stage of fruit maturity at the time of harvest and conservation of seed quality during storage. Santoso, Budianto and Aryana (2012) evaluated the germination of *J. curcas* seeds obtained from fruit at different stages of maturation and found greater storage potential for seeds obtained from fruit harvested in the yellow and brownish-yellow stages than seeds extracted from green and dry fruit. However, these seeds were stored for only six months and only one vigor test was performed on them, namely, a germination test.

Thus, the aim of this study was to evaluate the effect of the stage of fruit maturity on the germination and vigor of *J. curcas* seeds placed in Kraft paper bags and stored for 18 months in a laboratory environment.

MATERIALS AND METHODS

The experiment was conducted in the Seed Research Laboratory of the Plant Science Department at the Universidade Federal de Viçosa, Viçosa, Minas Gerais (MG), Brazil. *J. curcas* fruits were harvested in January 2012 in a commercial production area also in the municipality of Viçosa. Female flowers were tagged daily during the flowering phase of the plants, from the day of anthesis until enough fruit samples were obtained.

The fruits were harvested at different stages of maturity based on the color of the peel, ranging from yellow to brownish-yellow to completely brown (dry fruit), at approximately 60, 70, and 80 days after anthesis, respectively. The seeds were manually extracted from the fruit at each stage of maturity and dried naturally in a laboratory environment until reaching a degree of moisture compatible with storage, around 8%. After that, the seeds were placed in Kraft paper bags and stored in a laboratory environment for periods of 0, 3, 6, 9, 12, 15, and 18 months. The temperature and relative humidity of the environment were monitored daily with the aid of a thermohygrograph. After each period of storage of the seeds, their moisture content was determined and germination and vigor tests were performed.

Moisture content was determined by the oven method: 105 ± 3 ºC for 24 h (BRASIL, 2009). Four replications were used of around 10 g of seed each. Results were expressed in percentage.

The germination test was conducted with eight replications of 25 seeds placed in a roll of paper towell moistened with water weighing 2.7 times the weight of the dry substrate. The rolls were kept in a seed germinator at 25 ºC and the percentage of normal seedlings was calculated at seven and twelve days after sowing (OLIVEIRA *et al*., 2014a).

The first count of germination test was conducted together with the germination test. The result represented the percentage of normal seedlings obtained at seven days after sowing.

The accelerated aging test was conducted with eight replications of 25 seeds that were placed in a single layer on a metallic screen within a *gerbox* type box. Each box contained 40 mL of distilled water at the bottom. Lids were placed on the boxes to obtain internal relative humidity (RH) of 100% and kept in a BOD incubator at 42 ºC for

48 h (OLIVEIRA *et al*., 2014b). After this period, the seed germination test was performed. The result represented the percentage of normal seedlings at seven days after sowing.

The cold test was performed with eight replications of 25 seeds each. The seeds were placed on two sheets of paper toweling and covered with a fine layer of earth. After that, they were covered with a third sheet of paper toweling and rolls were made, which were kept in a BOD incubator at 10 ºC for nine days. After that, the rolls were transferred to a seed germinator at 25 ºC for five days, and then the percentage of normal seedlings was determined.

Four replications of 25 seeds were used to determine the electrical conductivity of the seed exudate. The seeds were weighed and placed in plastic cups containing 75 mL of distilled water (ISTA, 1995). The cups were then kept in a BOD incubator at 25 ºC for 24 h. After this period, electrical conductivity was determined in a Digmed DM-31 conductivity meter. Results were expressed in $\mu S\ cm^{-1}\ g^{-1}$ of seeds.

Seedling emergence was evaluated in four replications of 25 seeds that were sown in a substrate composed of earth and sand at a proportion of 2:1 in 1.5 L capacity polystyrene trays in a greenhouse. The substrate was first moistened until reaching 60% water retention capacity and then irrigated daily. The number of emerged seedlings were counted daily until 21 days after sowing, i.e., enough time for stabilization of counting. Seedlings with cotyledons above the surface of the substrate were considered emerged. The result was expressed as the percentage of seedlings that emerged up to 21 days after sowing.

The experiment was conducted in a completely randomized design in a split-plot arrangement with four replications. The plots contained fruit at different stages of maturity and the split-plots contained different storage periods. Analysis of variance was performed on the data. The mean values obtained for the stages of fruit maturity in each period of analysis were compared by the Tukey test ($P<0.05$). Regression analysis was performed on the data obtained in the storage periods for each stage of maturity. The estimates of the regression parameters were analyzed by the t-test ($P<0.05$).

RESULTS AND DISCUSSION

There was a significant interaction between the stage of fruit maturity and the storage period for all the tests of physiological quality of *J. curcas* seeds, except for germination and first count of germination (Table 1). However, all interactions were analyzed, regardless of significance, because the aim of the study was to investigate seed response at different stages of maturity during storage.

The data regarding temperature and relative humidity of the seed storage environment are shown in Table 2. The temperature values ranged from 19.7 ºC to 28.2 ºC, and relative humidity ranged from 49% to 77%. Maximum and minimum temperature and relative humidity readings were relatively stable throughout the storage period (23.2 ± 2.7 ºC; 64 ± 11% RH).

Seed conservation during storage is closely related to moisture content because this parameter affects seed chemical composition and the speed of seed metabolic activities (ALMEIDA *et al*., 2002). In this experiment, seeds were stored with moisture content near 8.0% after natural drying (Table 3). During storage, there were small variations in the values of seed moisture content in the three stages of fruit maturity, and a small increase was observed up to twelve months of storage, followed by a slight decline (Figure 1a, Table 4).

One factor in rapid loss of vigor in *J. curcas* seeds is high moisture content, and another is storage conditions (MONCALEANO-ESCANDON *et al*., 2013). According to Joker and Jepsen (2003), *J. curcas* seeds can be stored for at least a year, as long as they have low moisture content, from 5% to 10%. Results obtained by Worang *et al*. (2008) suggest that moisture contents from 7.9% to 8.4% are considered safe for storing seeds of this species under natural conditions (mean temperature of 26.5 ºC and 72% RH). Thus, the slight variation in seed moisture content observed in this experiment can be attributed to variations in the relative humidity and environmental temperature conditions (Table 2). Nevertheless, the values that were observed were within the limits considered adequate for conservation of these seeds. In addition, seed moisture content did not differ in the different stages of fruit maturity throughout the storage period (Table 3).

There was a small increase in germination values (Figure 1b, Table 4) and in first count of germination (Figure 1c, Table 4) of seeds obtained from brown and brownish-yellow fruit in the first nine months of storage. This can be attributed to reduction in the proportion of hard seeds, which was initially 5% and 8%, and was reduced to 2% and 3% at nine months of storage for the brown and brownish-yellow stages, respectively. The seeds from yellow fruit practically maintained their initial values until the ninth month of storage.

The germination and first count of germination values obtained for the three stages of fruit maturity were initially similar and remained so throughout the storage period (Table 3). These results are consistent with those obtained by Silva *et al*. (2012), who found similar

Table 1 - Summary of analysis of variance of the physiological quality parameters of *J. curcas* seeds during storage in accordance with the stage of fruit maturity

SV	DF	Mean Square						
		MC	GER	FCG	AE	CT	EC	EME
ST	2	0.062^{ns}	62.7^{ns}	0.15^{ns}	79.9^{ns}	474.5*	12582.0*	269.1*
Residue a	9	0.021	66.4	79.54	37.9	23.5	3.8	39.2
PER	6	4.112*	1483.1*	1929.28*	3148.6*	4356.2*	1347.8*	2376.0*
ST*PER	12	0.265*	50.7^{ns}	56.14^{ns}	73.9*	220.8*	32.2*	208.2*
Residue b	54	0.061	31.1	40.65	9.09	22.3	4.7	23.1
CV (%)		2.85	6.59	7.93	3.89	9.28	2.07	7.58

SV - Source of variation; ST - Stage of fruit maturity; PER - Period of storage; MC - Moisture content; GER - Germination; FCG - First count of germination; AE - Accelerated aging; CT - Cold test; EC - Electrical conductivity; EME - Emergence; ns - not significant, * - significant at 5% probability by the F test

Table 2 - Amplitude of temperature and relative humidity in the environment where *J. curcas* seeds were stored

Storage (months)	Temperature (°C)	Relative Humidity (%)
0-3	21.7 - 24.1	65.9 - 77.2
3-6	19.7 - 23.0	51.8 - 69.0
6-9	22.2 - 26.2	54.1 - 70.7
9-12	23.8 - 28.2	57.0 - 71.8
12-15	21.9 - 27.0	54.9 - 71.0
15-18	20.2 - 25.4	49.0 - 66.9

Table 3 - Moisture content, germination, first count of germination, accelerated aging, cold test, electrical conductivity, and emergence of seedlings of *J. curcas* seeds during storage in accordance with the stage of fruit maturity[(1)]

Stage	Storage (months)						
	0	3	6	9	12	15	18
	Moisture content (%)						
Brown	7.8 a	8.5 a	8.3 a	9.6 a	9.7 a	8.7 a	8.0 a
Brownish-yellow	8.4 a	8.5 a	8.5 a	8.9 b	9.7 a	8.9 a	8.2 a
Yellow	8.0 a	8.4 a	8.4 a	8.7 b	9.5 a	9.3 a	8.1 a
CV (%)	2.85						
	Germination (%)						
Brown	87 a	88 a	91 a	95 a	81 a	77 a	62 a
Brownish-yellow	88 a	89 a	94 a	93 a	89 a	85 a	58 a
Yellow	93 a	94 a	94 a	88 a	82 a	86 a	64 a
CV (%)	6.59						
	First count of germination (%)						
Brown	85 a	87 a	86 a	91 a	84 a	70 a	60 a
Brownish-yellow	87 a	85 a	91 a	89 a	88 a	73 a	51 a
Yellow	92 a	89 a	91 a	85 a	78 a	74 a	54 a
CV (%)	7.93						

Continued Table 3

	Accelerated aging (%)						
Brown	85 a	83 a	85 a	89 a	84 a	66 a	38 b
Brownish-yellow	78 a	88 a	91 a	94 a	80 a	70 a	47 ab
Yellow	86 a	87 a	94 a	86 a	76 a	73 a	49 a
CV (%)	3.89						
	Cold test (%)						
Brown	60 a	72 a	60 a	65 a	50 a	28 a	15 b
Brownish-yellow	68 a	74 a	67 a	74 a	44 ab	32 a	30 a
Yellow	58 a	78 a	64 a	42 b	35 b	32 a	23 ab
CV (%)	9.28						
	Electrical conductivity (μS cm^{-1} g^{-1})						
Brown	115.5 a	121.7 a	126.5 a	129.5 a	123.9 a	126.9 a	152.9 a
Brownish-yellow	89.6 b	92.2 b	95.3 b	101.5 b	93.7 b	104.3 b	120.0 b
Yellow	73.6 c	75.7 c	86.0 c	92.5 c	84.8 c	90.8 c	103.5 c
CV (%)	2.07						
	Seedling emergence (%)						
Brown	84 a	76 a	59 a	72 a	38 c	53 a	38 a
Brownish-yellow	83 a	75 ab	58 a	80 a	54 b	55 a	47 a
Yellow	88 a	62 b	56 a	74 a	68 a	63 a	50 a
CV (%)	7.58						

[(1)]Mean values followed by the same letter in the column for each test do not differ from each other by the Tukey test (P<0.05)

germination values in seeds obtained from fruit in the yellow, brownish-yellow, and brown stages evaluated soon after harvest. However, Dranski *et al*. (2010) found that fruit senescence led to a reduction in germination, i.e., the germination of seeds from dry fruit was less than that of seeds from yellow and brownish-yellow fruit.

From the ninth month of storage on, there was a reduction in both germination and in first count of germination of seeds from the three stages of fruit maturity (Figures 1b, c).

At 18 months of storage, germination reduced 25, 30, and 29 percentage points in seeds from the brown, brownish-yellow, and yellow fruit stages, respectively, in relation to the values observed at the beginning of storage (Figure 1b, Table 4).

Pereira *et al*. (2013) were able to detect differences in the physiological quality of *J. curcas* seeds stored under different environment and packaging conditions more clearly from nine months of storage on. Worang *et al*. (2008) observed that *J. curcas* seeds with 89% germination that were stored at ambient conditions (mean of 26.5 ºC and 72% RH) in plastic bags had germination of 75% after one month of storage 53% after sixth months. In addition, Guzman and Aquino (2009) observed total loss of germination of *J. curcas* seeds stored for 12 months under conditions without any control.

Santoso, Budianto and Aryana (2012) evaluated germination of *J. curcas* seeds while they were stored in plastic bags under laboratory conditions and observed higher percentages of germination for seeds from yellow and brownish-yellow fruit compared to germination obtained for seeds from brown fruit (dry), from the first month of storage on.

The accelerated aging test also showed reduction in seed viability (Figure 1b, Table 4) and vigor (Figure 1c, Table 4) and a decline in speed of germination over the storage period (Figure 1d, Table 4). Aged seeds revealed a small increase in germination up to the third month of storage and more expressive reduction in vigor from the ninth month of storage on, regardless of the stage of fruit maturation. These results are consistent with those obtained by Oliveira (2013), who observed a linear decline in germination in the accelerated aging test of *J. curcas* seeds stored for one year under ambient conditions (mean of 23 ºC and 75% RH).

The values obtained in the accelerated aging test were similar for the different stages of fruit maturity throughout the storage period (Table 3), except for 18 months of storage, when seeds extracted from yellow fruit had greater vigor than those obtained from brown fruit.

In the cold test, the vigor of seeds obtained from yellow and brownish-yellow fruit declined in a linear manner over the storage period (Figure 1e, Table 4). In contrast, the vigor of seeds from brown fruit declined only from the ninth month of storage on. These results corroborate those obtained by other authors for seeds of this species stored under conditions similar to those of this study (OLIVEIRA, 2013; PEREIRA *et al*., 2013;); this shows reduction in seed vigor due to the process of deterioration during storage.

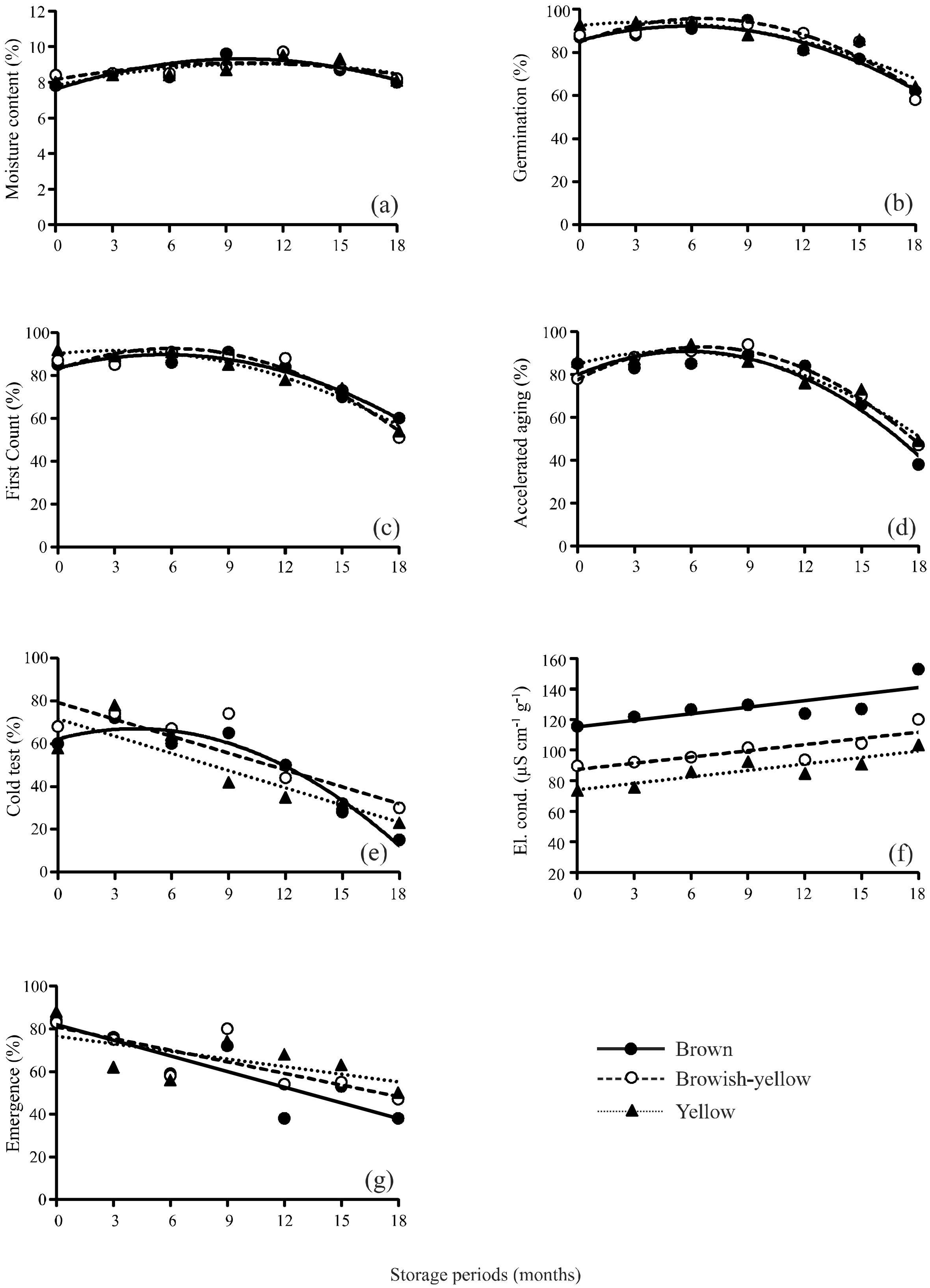
Seeds obtained from yellow fruit at nine and twelve months of storage showed less vigor (Table 3). However, with the expressive decline in seed vigor, this difference was not maintained. At the end of the storage period, i.e., at 18 months, seeds extracted from brownish-yellow fruit showed higher germination in the cold test; this germination percentage was similar to that obtained for seeds extracted from yellow fruit.

Table 4 - Equations fitted to the data obtained in determination of moisture content and in tests of germination, first count of germination, accelerated aging, cold test, electrical conductivity, and seedling emergence of *J. curcas* L. seeds harvested at different stages of fruit maturity in accordance with the storage period

Stage	Equation	R^2
	Moisture content (%)	
Brown	$Y = 7.61607 + 0.3494^{*}X - 0.01786^{*}X^2$	0.71
Brownish-yellow	$Y = 8.13494 + 0.19958^{*}X - 0.01021^{*}X^2$	0.46
Yellow	$Y = 7.81393 + 0.23223^{*}X - 0.01076^{*}X^2$	0.59
	Germination (%)	
Brown	$Y = 85.42857 + 2.35714^{*}X - 0.20238^{*}X^2$	0.94
Brownish-yellow	$Y = 84.75 + 3.3631^{*}X - 0.25595^{*}X^2$	0.89
Yellow	$Y = 92.47619 + 0.91964^{ns}X - 0.12731^{*}X^2$	0.84
	First count of germination (%)	
Brown	$Y = 83.36310 + 2.23214^{*}X - 0.19709^{*}X^2$	0.94
Brownish-yellow	$Y = 82.82738 + 3.23214^{*}X - 0.26852^{*}X^2$	0.93
Yellow	$Y = 90.27381 + 0.93155^{ns}X - 0.15642^{*}X^2$	0.96
	Accelerated aging (%)	
Brown	$Y = 79.78571 + 3.83929^{*}X - 0.33135^{*}X^2$	0.93
Brownish-yellow	$Y = 77.5119 + 4.6607^{*}X - 0.3525^{*}X^2$	0.98
Yellow	$Y = 84.77381 + 2.40476^{*}X - 0.23677^{*}X^2$	0.94
	Cold test (%)	
Brown	$Y = 61.60714 + 2.43452^{*}X - 0.28770^{*}X^2$	0.95
Brownish-yellow	$Y = 78.83929 - 2.60119^{*}X$	0.75
Yellow	$Y = 71.52679 -2.68155^{*}X$	0.78
	Electrical conductivity (µS cm^{-1} g^{-1})	
Brown	$Y = 115.22098 + 1.43152^{*}X$	0.62
Brownish-yellow	$Y = 87.32848 + 1.35521^{*}X$	0.71
Yellow	$Y = 73.99027 + 1.41176^{*}X$	0.80
	Seedling emergence (%)	
Brown	$Y = 81.96429 - 2.45238^{*}X$	0.75
Brownish-yellow	$Y = 80.74107 - 1.8125^{*}X$	0.66
Yellow	$Y = 76.58929 - 1.19643^{*}X$	0.38

^{ns} - not significant, * - significant by the t-test at 5% probability

Figure 1 - Moisture content (a), germination (b), first count of germination (c), accelerated aging (d), cold test (e), electrical conductivity (f), and emergence of seedlings (g) of *J. curcas* L. seeds harvested at different stages of fruit maturity in accordance with the storage period



The values of electrical conductivity increased with the increase in the storage period of seeds for the three stages of fruit maturity (Figure 1f, Table 4). An increase in electrical conductivity is associated with the accumulation of prejudicial changes in the cell membranes resulting from the process of seed deterioration over the storage period (BASRA; REHMAN; IQBAL, 2000; MONCALEANO-ESCANDON *et al*., 2013). Thus, the electrical conductivity test can identify physiological and biochemical changes and is related to change or loss of integrity of the cell membrane system (DELOUCHE; BASKIN, 1973). Low values of electrical conductivity are related to greater organization of cell membranes and, consequently, greater seed vigor.

Other authors also observed an increase in the values of electrical conductivity of *J. curcas* seeds over storage (CHAVES *et al*., 2012; OLIVEIRA, 2013), showing loss of integrity of the cell membrane system due to the seed deterioration process (DELOUCHE; BASKIN, 1973) and, as a consequence, loss of physiological quality. Thus, reduction in germination (Figure 1b, Table 4) and decline in vigor of *J. curcas* seeds (Figures 1c, d, e, Table 4) occurred along with the increase in values of electrical conductivity obtained in this experiment (Figure 1f, Table 4).

Lower values of electrical conductivity were obtained for seeds extracted from yellow fruit in all storage periods, indicating greater vigor in relation to seeds from the other stages of maturity, whereas lower vigor was observed in seeds from brown fruit (Table 3). These results corroborate those obtained by Silva *et al*. (2012), who found lower electrical conductivity in newly harvested seeds obtained from yellow fruit compared to those from brownish-yellow and brown fruit.

The percentage of seedling emergence declined over the storage period (Figure 1g, Table 4), which is consistent with results obtained by Oliveira (2013) and Worang *et al*., (2008) for *J. curcas* seeds. This decline was less accentuated for seeds extracted from yellow fruit, which reveals slower reduction in the vigor of these seeds in comparison to seeds of the other stages of maturity.

When the three stages of fruit maturity are compared, differences are observed in seedling emergence only at three and twelve months of storage (Table 3). At three months of storage, higher values of emergence were obtained for seeds extracted from brown and brownish-yellow colored fruit, whereas at twelve months of storage, the seeds extracted from yellow fruit had a higher percentage of seedling emergence. However, in the longest periods of storage, that is, at 15 and 18 months, seeds extracted from yellow fruit tended to have higher values of emergence, although they did not differ statistically from seeds from the other stages (Table 3).

In short, germination percentage of *J. curcas* seeds declined from the ninth month of storage on, regardless of the stage of fruit maturity (Figure 1b, Table 4). However, a significant difference in percentage of seed germination among the three stages of fruit maturity was not observed throughout the storage period (Table 3). The tests of first count of germination (Figure 1c, Table 4), accelerated aging (Figure 1d, Table 4), and cold test for seeds from brown fruit (Figure 1e, Table 4) show reduction in seed vigor at all stages of fruit maturity, especially from nine months of storage on.

The decline in vigor was less intense for seeds extracted from yellow and brownish-yellow fruit compared to seeds obtained from brown fruit, which was clearly shown by seedling emergence, electrical conductivity, and cold tests (Table 3).

CONCLUSIONS

1. *J. curcas* seeds can be stored for up to nine months without significant loss in germination and vigor, and for up to twelve months with a high percentage of germination, though with a decline in vigor;
2. Seeds extracted from yellow and brownish-yellow colored fruit have greater vigor and maintain physiological quality for a longer time compared to seeds extracted from brown fruit, i.e., dry fruit.

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