Heat treatment and germination of seeds of interspecific hybrid between American oil palm (*Elaeis oleifera* (H.B.K) Cortes) and African oil palm (*Elaeis guineensis* Jacq.)¹

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ABSTRACT – The oil palm (*E. guineensis*) is the African origin and the world's leading source of vegetable oil. The interspecific hybridization of the African oil palm (*E. guineensis*) with American oil palm (*E. oleifera*) aims to improve resistance to diseases, to improve oil quality and lower plant height. EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária, Brazilian Corporation of Agricultural Research) has developed the first Brazilian interspecific hybrid cultivar (HIE) between American oil palm and African oil palm. The procedures adopted for commercial seed germination assessment have shown an average germination rate of 32%. The objective of this work was to assess the effect of the period of heat treatment and seed water content that are ideal for breaking dormancy and obtaining maximum germination. A completely randomized design was adopted, in a 4 x 3 factorial design, with four ranges of moisture contents: 18-19; 19-20; 20-21 and 21-22%, and three periods of heat treatment: 55, 75 and 100 days, with three replicates of 500 seeds. The percentage of germination, the first count and the germination speed index were assessed. To break dormancy and germination, the hybrids seeds of HIE, *oleifera* versus *guineensis*, should have their water content adjusted to values between 19 and 22%, and be subjected to heat treatment at a temperature of 39 ± 1 °C and relative humidity of approximately 75% for 75 days.

Index terms: Elaeis guineensis, Elaeis oleifera, oil palm, breaking dormancy.

Tratamento térmico e germinação de sementes do híbrido interespecífico entre o caiaué e o dendezeiro

RESUMO - O dendezeiro, palmeira de origem africana, representa a principal fonte mundial de óleo vegetal. A hibridação interespecífica do dendezeiro com o caiaué, de origem americana, tem como objetivos a resistência a doenças, melhoria na qualidade do óleo e menor altura de plantas. A Embrapa desenvolveu a primeira cultivar brasileira híbrida interespecífica (HIE) entre o caiaué e o dendezeiro. Os procedimentos adotados para avaliar a germinação das sementes comerciais dessa cultivar têm apresentado valores médios de 32%. O objetivo desse trabalho foi avaliar o efeito do período de tratamento térmico e do teor de água nas sementes, ideais para superação da dormência e obtenção da germinação máxima. Adotou-se o delineamento inteiramente casualizado, em esquema fatorial 4 x 3, sendo quatro intervalos de graus de umidade: 18-19; 19-20; 20-21 e 21-22% e três períodos de tratamento térmico: 55, 75 e 100 dias, com três repetições de 500 sementes. Foram avaliadas a porcentagem de germinação, a primeira contagem e o índice de velocidade de germinação. Para superação da dormência e germinação, as sementes híbridas do HIE: *oleifera* x *guineensis* devem ter seu teor de água ajustado para valores entre 19 e 22% e ser submetidas ao tratamento térmico sob temperatura de 39 ± 1 °C e umidade relativa do ar de aproximadamente 75% por 75 dias.

Termos para indexação: Elaeis guineensis, Elaeis oleifera, palma de óleo, superação de dormência.

Introduction

The oil palm (*Elaeis guineensis* Jack.) is of African origin and the world's leading source of vegetable oil (USDA, 2014). American oil palm (*Elaeis oleifera* H.B.K. Cortés), a semidomesticated species of South American origin, is from the same genus as the African oil palm, but has no importance in itself in the commercial production of oil. These two species can be crossed with ease and interspecific hybridization has been used for genetic improvement, seeking to associate the

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high productivity of the African oil palm with characteristics of the American oil palm, such as smaller height, improved oil quality (greater unsaturation) and mainly resistance to diseases, particularly to fatal yellowing (FY) (Cunha et al., 2012), a disease of unknown etiology that has already claimed thousands of hectares of plantations of the African species (Boari, 2008).

From studies started in the 1990s, the first national interspecific hybrid cultivar was developed by Embrapa between American oil palm and African oil palm (HIE OxG), called BRS Manicoré, which, in addition to a high productivity, is resistant to FY, presents a reduced height growth and a more unsaturated oil than the tenera cultivars (intraspecific hybrid) of the African species (Cunha and Lopes, 2010).

In commercial seed production of interspecific hybrid, BRS Manicoré has been showing low rates of germination, about 30 to 35%, according to a personal communication from the business office Escritório de Negócios da Amazônia -Embrapa Produtos e Mercado. Increasing the germination rate of HIE OxG reduces the cost of production and, consequently, the cost of selling to the producer, as well as increasing the supply capacity of germinated seeds of this material, without expanding fields of seed production. According to Guerrero et al. (2011), to the arrival of new OxG cultivars are added new problems and among them stands out the low germination of these seeds. The authors have reported that four Colombian producers: Hacienda La Cabaña, Meta; Unipalma, Meta; Indupalma, Cesar y Corpoica El Mira, Nariño and Embrapa report germination rates of HIE OxG below 30%.

According to Hussey (1958), the African oil palm seeds have low germination rates due to the dormancy that they present after physiological maturity, caused by the mechanical resistance of the endocarp, which has a hard and dense consistency, and the absorption of oxygen, preventing the growth of the embryo. Under natural conditions, as is common in the *Elaeis* genus, germination can take years and is generally non-uniform and low. Studies related to commercial seed germination of African oil palm were developed by Hussey (1958) and Rees (1962), who observed the need to subject seeds to heat treatment to break dormancy. Since then, several procedures have been developed for germination of African oil palm seeds based on the thermal treatment (heating) to break dormancy.

For germination of seeds of HIE OxG, Embrapa has used the method described by Corrado and Wuidart (1990), which consists, in the storage of seeds with moisture content between 7-10% (fresh weight of almond); hydration of seeds by soaking in water for seven days; heat treatment of the seeds in Heat Room Seed Germinator at a temperature from 39 to 40 °C, for 100 days; and at the end, a new hydration for seven days and then seed germination at a temperature of about 27 °C. This process requires approximately 150 days and the results are not satisfactory, because the germination percentage obtained is low.

New materials have been produced by research to meet the different demands of national African oil palm culture, including HIEs, families of American oil palm and of African oil palm selected in germination germplasm bank. Simultaneously, the production, storage and germination procedures should be improved so that the demand of the farmers for the seeds of the new materials are met in terms of quality and quantity.

The aim of this study was to determine the water content of the seeds and the period of optimal heat treatment to break dormancy and germination of seeds of HIE OxG BRS Manicoré.

Material and Methods

The experiment was conducted at Embrapa Amazônia Ocidental, Campo Experimental do Rio Urubu (CERU), Rio Preto da Eva, AM (2°27'08.44" S, 59°34'13.69" W) and in the laboratory Laboratório de Dendê e Agroenergia, km 29 da Rodovia AM, in Manaus, AM.

Seeds of cultivar BRS Manicoré (Cunha and Lopes, 2010) were used, interspecific hybrid between species *E. oleifera* (origin: Manicoré) and *E. guineensis* (origin: La Mé), produced by Embrapa Amazônia Ocidental. The seeds were produced by controlled pollination, and the bunches were harvested when they had three to five mature fruits naturally detached from the bunch (physiological maturity). After harvesting, the fruits were manually removed from the bunch and mesocarp was extracted in an electric centrifugal depulper. Then the seeds (endocarp, endosperm and embryo) were dried eliminating the ones deformed or damaged by processing, performing a fungicide treatment and then homogenization of seeds and formation of lots to be used in the study.

A completely randomized design was adopted, with three replications of 500 seeds for each treatment, in a 4 x 3 factorial design, with four ranges of moisture content of the seeds and three periods of stay in the Heat Room Seed Germinator (heat treatment). The periods of stay of seeds in the heat treatment (39 ± 1 °C and relative humidity of approximately 75%) were of 55, 75 and 100 days and the range of seed moisture contents of 18 to 19%; 19 to 20%; 20 to 21% and 21 to 22%. The determination of moisture content of the seeds was performed by the oven method at 105 °C ± 2 °C, for 24 hours, according to Brasil (2009), using as sampling four replicates of 10 seeds for each lot of 500 seeds. The ranges of moisture content were obtained and set using the formulas:

$$\begin{split} U(\%) &= \left[\frac{(A_1 - A_2)}{A_2}\right] \times 100 \,; \\ U_m(\%) &= \frac{U_1(\%) + U_2(\%) + U_3(\%) + U_4(\%)}{4} \,; \\ PS &= \left[\frac{PFT}{(100 + U_m\%)}\right] \times 100 \,; \\ U_T(\%) &= \left[\frac{P}{PS - 1}\right] \times 100 \,; \end{split}$$

where:

U(%) = moisture content of the treatment;

 A_1 = initial weight of the moist sample, in grams;

 A_2 = final weight of the dry sample (after 24 h in the oven), in grams;

 U_{m} (%) = average moisture content of the treatment;

 U_1 (%), U_2 (%), U_3 (%), U_4 (%) = moisture contents of samples 1, 2, 3 and 4, respectively;

PS = original dry weight of the treatment;

PFT = fresh weight of the treatment;

 U_{T} (%) = present moisture of the seeds of the treatment.

After adjustment of the moisture content, the seeds of each moisture range were packed in polyethylene bags of 65 x 50 cm and a thickness 0.2 mm, sealed, containing air volume at least equal to the volume of seeds and placed in a Heatbox Seed Germinator (heating chamber controlled by electric resistance, system of digital temperature setting, forced air circulation and monitoring of relative humidity) to be submitted to different periods of heat treatment.

At the end of each period of thermal treatment, the seeds were rehydrated by immersion in water tanks, under oxygen for eight days. Then, the seeds were placed in polyethylene bags (65 cm x 50 cm, thickness 0.2 mm), tightly closed and kept for germination in a room with controlled temperature between 27 e 30 °C.

The count of germinated seeds was performed four times,

the first being held 15 days after preparation of the seeds in the germination room and the others weekly. The seed with visible protrusion of the hypocotyl-radicle axis was considered germinated. To calculate the percentage of germination, the seeds discarded by contamination and non-germinated were considered as non-germinated. At 15 days the first count was assessed (FC) and after 35 days the percentage of germination was assessed (GERM). At the end of the germination period, the Emergence Speed Index (ESI) was calculated using the formula described by Nakagawa (1999), with adaptations:

$$ESI = G_1/N_1 + G_2/N_2 + ... + G_n/N_n$$

where:

 $G_n =$ number of germinated seeds in the nth week. $N_n = n^{th}$ assessment week of germination.

The data for these variables was tested using the Lilliefors normality test and analysis of variance and means were compared by Tukey test (5 % probability). Statistical analyses were performed using the software ASSISTAT (Silva and Azevedo, 2002).

Results and Discussion

All variables were normally distributed, with the original data submitted to analysis of variance (Table 1). Significant effects of the factors of the period of stay in a heat treatment and moisture content of the seeds were observed, as well as the interaction among them in the variables related to FC and ESI force. In the GERM variable, the effects of the factors were significant and the interaction among them was not significant. The results show that there is a complex interaction in the response of FC and ESI, and the simple effects of the factors should be analyzed, i.e., for each residence time in the heat treatment the answer should be analyzed in relation to changes in moisture content, and vice versa. In the case of GERM, as the interaction effect was not significant, the main effects of the factors were analyzed.

 Table 1.
 Summary of analysis of variance of germination (GERM), first count (FC) and emergence speed index (ESI) assessed for cultivar BRS Manicoré seeds.

SV	DE	GERM		FC		ESI	
	Dr =	AS	F	AS	F	AS	F
Heat treatment (F1)	2	1532.97	24.22**	814.68	13.14**	277.80	7.89^{**}
Moiture content (F2)	3	3403.46	53.77**	6048.84	97.55**	1955.36	55.57**
Interaction F1 x F2	6	77.52	1.22 ^{ns}	317.95	5.13**	120.67	3.43**
Treatment	11	13741.47	19.74**	1971.23	31.79**	649.61	18.46**
Residue	24	63.29		62.01		35.19	
Mean		60.94		39.94		25.42	
CV (%)		13.05		19.71		23.33	

ns – non significant (p \geq 0.05); ** significant at 1 % probability by the F test; SV – Source of variation; F1 – Factor 1; F2 – Factor 2; DF – Degree of freedom; CV – Coefficient of variation; AS – Average square.

The germination of seeds subjected to 55 days of stay in the heat treatment was lower than that obtained in periods of 75 and 100 days, which did not differ statistically with each other (Table 2). The results indicate that the period of 75 days of treatment was sufficient to break dormancy and promote a high germination rate of seeds (68%). Seeds with 21-22% moisture had a germination of 76%, a value with no statistical difference of the 72% of germination of seeds with 20-21% moisture. In the range of 19-20% moisture, seed germination was 63% and did not differ statistically from seed germination with 20-21% moisture (72%). Seed germination with 18 to 19% (33%) was lower than the other treatments. The results indicate that to obtain the highest rates of germination seed, moisture content should be between 21 and 22%.

In Figure 1, it can be seen that in the heat treatment period of 75 days after germination of seeds from treatments with higher contents of moisture (19-20%, 20-21% and 21-22%), it ranged from 71.1 to 84.9%, values that did not differ statistically with each other and were superior to the interval with lower moisture content (18-19%). Similar behavior was obtained for 100 days of heat treatment, i.e., the range of moisture 18-19% was statistically inferior to the others, and

the values of germination of seeds with higher moisture were not statistically different from each other. It was also found that the germination rates obtained in the three upper ranges of moisture content were not statistically different between the periods of heat treatment of 75 and 100 days.

Table 2. Germination (%) of seeds of the interspecific hybrid between *Elaeis oleifera* and *E. guineensis* (cultivar BRS Manicoré) according to the period of heat treatment (39 °C \pm 1 °C) and moisture content of the seeds.

Heat treatment period (days)	Germination (%)			
55	48 b			
75	68 a			
100	67 a			
Moisture content of seeds (%)	Germination (%)			
18-19	33 c			
19-20	63 b			
20-21	72 ab			
21-22	76 a			

Means followed by the same letter in the column do not differ among themselves by the Tukey test at 5% probability.



Figure 1. Germination (%) of seeds of BRS Manicoré in different ranges of moisture content (18-19%, 19-20%, 20-21% and 21-22%), subjected to periods of stay in the heat treatment of 55, 75 and 100 days. Means followed by equal uppercase letters within the periods of stay, and by equal lowercase letters within the same range of moisture, do not differ with each other by the Tukey test (p < 0.05).

To maximize the germination process of commercial seeds, one must consider the percentage of germination obtained with the combination of different treatments and costs, mainly energy spent for heating and maintaining the heat room and specific worker for weekly reviews of lots (exchange air from the bags, check incidence of fungi, assessment of germination). Considering these aspects, as no significant differences between the germination of the seeds were observed in three ranges of higher contents of moisture and between the periods of heat treatment of 75 to 100 days, the minimum time should be used, i.e., 75 days, by adjusting seed moisture content to values between 19-22 %, conditions in which germination values higher than 71% were obtained. The water content of the seeds to be subjected to heat treatment is directly related to the probability of incidence of fungi, since the seeds are exposed for more than two months at ambient temperature and relative humidity conducive to growth of microorganisms, namely, 39 °C and 75%, respectively. In the process of these seeds after heat treatment, weekly assessments are conducted in order to separate and count the germinated seeds. Information obtained in these counts can be used to calculate the speed of germination, which are related to seed vigor.

Significant differences between FC and ESI variable averages were observed, by the effect of moisture and by the heat treatment time (Figures 2 and 3). Significantly higher values for FC and ESI were observed in the highest ranges of moisture, especially during the heat treatment of 75 and 100 days, in which the averages for the maximum range of moisture (21-22%) were higher than the other treatments and did not differ statistically with each other.



Figure 2. Germination in the first count (%) of seeds of BRS Manicoré in different ranges of moisture content (18-19%, 19-20%, 20-21% and 21-22%), subjected to periods of stay in the heat treatment of 55, 75 and 100 days. Means followed by equal uppercase letters within the periods of stay, and by equal lowercase letters within the same range of moisture, do not differ with each other by the Tukey test (p < 0.05).

Methods for rapid and uniform germination of African oil palm seeds, in commercial scale, go back to the middle of the last century, but there is still the need to investigate new practices and/or techniques involved in the germination process, taking into account the retelling of the period required for heat treatment to break dormancy with the market launch of genetically distinct materials. Recent example is the work done by Green et al. (2013), who studied the effect of heating periods (40, 50, 60 e 80 days) on the germination of six cultivars of tenera of African oil palm Deli x La Mé produced by Embrapa, in which the results showed different responses, depending on the cultivar, and the maximum value can be obtained germination between 45 and 80 days of heat treatment, with varying percentages of germination of 70-92%. Fondom et al. (2010), also analyzing the germination of seeds of ten progenies of African oil palm, found that seeds with moisture content of 18% germinated better when subjected to 39 ± 1 °C for 60 days, compared to periods of 80, 100 and 120 days.

Even with the growing demand for HIEs, especially in Latin America, most of the required methodology for germination of these hybrids are still based on procedures for the African oil palm. The specific studies on the germination of HIE OXG are recent. Guerrero et al. (2011) assessed the effect of varying moisture content of the seeds at the beginning of the heat treatment and different periods of heat treatment. These authors have obtained better results with seeds with 20% and 22% moisture and heat treatment for 70 days at 39 °C, reaching an average germination of 40.4 and 36.3%, respectively, which are lower values compared to the germination of hybrid seeds of HIEs BRS Manicoré in this study, where germination above 70% was obtained. The authors have attributed the low percentage of germination to endogenous factors (sterility and immature embryo), mainly because it is an artificial cross between two different species and exogenous factors (latency and chemical inhibitors) that induce prolonged latency of seeds.



Figure 3. ESI of seedlings of BRS Manicoré in different ranges of moisture content (18-19%, 19-20%, 20-21% and 21-22%), subjected to periods of stay in the heat treatment of 55, 75 and 100 days. Means followed by equal uppercase letters within the periods of stay, and by equal lowercase letters within the same range of moisture, do not differ with each other by the Tukey test (p < 0.05).

The study by Guerreiro et al. (2011) was developed with the same limits of moisture content when entering in the heat treatment as in the present study, of 18 % and 22%; however, dividing the treatments into three moisture levels (18, 20 and 22%), whereas in the present four ranges of moisture were used (18-19%, 19-20%, 20-21% and 21-22%). Guerrero et al. (2011) recommended in their study that moisture levels should be explored next to the ones where they have obtained the best results, 20-22%, and in this range of seed moisture the highest values of germination were obtained, in this study. With respect to the period of stay of the seeds in the heat treatment, Guerrero et al. (2011) used five treatments (60, 70, 80, 90 and 100 days) and obtained a better germination in 70 days, an amount next to the one identified as the best treatment in this study, of 75 days. It is noteworthy that the female progenitors matrices (American oil palm) of the two studies are of different origins, Colombian in the case of Guerreiro et al. (2011) and Brazilian in this study. As a certainty, the results obtained by Guerreiro et al. (2011) and the one obtained in this study show that it is possible to increase the rate of germination of HIEs, reduce spending on the germination process and optimize the use of facilities by testing combinations of exposure time and moisture content of the seeds in the treatment of dormancy break.

Conclusions

To break dormancy and germination of hybrid seeds of HIE OxG BRS Manicoré, seeds with water content adjusted to values between 19 and 22% should be used, and heat treatment must be conducted at temperatures of 39 ± 1 °C and relative humidity of approximately 75% for 75 days.

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