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Full Length Research Paper

Effect of storage and heat treatments on the germination of oil palm (*Elaeis guineensis* Jacq.) seed

Beugré Manéhonon Martine[1], Kouakou Kouakou Laurent[1], Bognonkpé Jean Pierre[1], Konan kouakou Eugène[2], Kouakou Tanoh Hilaire[1]and Kouadio Yatty Justin[1]

[1]Laboratoire de Biologie et Amélioration des Productions Végétales, UFR des Sciences de la Nature, Université d'Abobo-Adjamé, 02 BP 801 Abidjan 02, Côte d'Ivoire.

[2]Programme Palmier, Laboratoire de culture in vitro, CNRA Station La Mé, 13 BP 989 Abidjan 13, Côte d'Ivoire.

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Oil palm (*Elaeis guineensis* Jacq.) is a perennial tropical tree which reproduces only by seeds. In the nature, the seeds have a relative dormancy leading to very long germination process (1 to 3 years). In order to improve oil palm production, research aiming at the reduction of the dormancy period was initiated. The germination tests carried on seeds stored for different periods after harvest, showed a germination percentage of fresh seeds (not stored) significantly higher (55.39 %) than that of seeds stored for 3 months (45.78%) and 6 months (38.68%). This germination ability also varies significantly with the two palm genotypes (C70XX from LM 19954 and C24XX from linear LM 19617) developed by the Centre National de Recherche Agronomique (CNRA) in Côte d'Ivoire and used in the present study. Seeds of category C70XX from LM 19954 linear present a significant superior percentage of germination than those of the category C24XX from linear LM 19617. In order to break seeds dormancy, heat incubation at 40°C was applied for different durations (40, 60 and 80 days). Seeds incubated for 60 days at 40°C induced higher germination percentage (56.3%) compared to the current applied delay of 80 days heating. 40 days heating period appeared to reduce the germination capability. According to these results, the usual germination techniques for oil palm seedlings production can be improved by shortening germination delay of seeds.

Key words: Oil palm, *Elaeis guineensis* Jacq., seed, storage, heating, germination.

INTRODUCTION

To ensure the reproduction of plants species, seeds or vegetative organs must germinate; that is begin metabolism activities to give a new plant (Côme and Corbineau, 1998). This process starts with seed imbibition witch lead to the embryo development (Bewley and Black, 1994). Seed germination, however, differs widely from one species to another, depending on the type of dormancy caused by various factors (Bewley 1997; Bewley and Black, 1994; Koomneef et al., 2002). Dormancy may be due to the embryo immaturity (Berjak et al., 1984, Yang et al., 2007; Baskin et al., 1998), the impermeability to the seed coat (Robertson and Small, 1977; Daquinta et al., 1996) or some physiological events that occurred in the seed during its storage (Yuri, 1987; Baskin et al., 2000; Leubner-Metzger, 2005; Bove et al., 2006; Oracz et al., 2007). Several techniques are used, especially for crops, to break seed dormancy and speed up the germination. Mechanical scarification and seeds treatment with gibberellin (GA_3), potassium nitrate (KNO_3) and hydrogen peroxide (H_2O_2) accelerate seed germination of *Areca trianda* and two rattan species: *Laccosperma secundiflorum* and *Eremospatha macrocarpa* (Yang et al., 2007; Cirak et al. 2007; Kouakou et al., 2009). Germination of certain species of the genus *Pinus* and *Citrus* is significantly increased after seed soaking in a solution of ash or with their exposure to high temperature, between 70 to 110°C (Herrero et al., 2007).

Among industrial plants cultivated in the world, oil palm (*Elaeis guineensis* Jacq.) has a prominent part of the volume of international trade, due to a ceaselessly growing demand of palm oil in the world. With more than 8 million of cultivated hectares in the world, oil palm is, after soybean, the second global source of vegetable oil

*Corresponding author. E-mail: tanohilaire@yahoo.fr

(Jacquemard, 1995) . In natural conditions, oil palm seed germination is very slow (1 to 3 years) with a weak percentage. These seeds are thus ranged among recalcitrant seeds, according to Braun (1984) and Chin et al. (1984). This slow and weak capacity of oil palm seed germination represents a major constraint for the establishment of plantings (Rees, 1963).

For a sustainable supply of seedlings to farmers, techniques to speed up seed germination have been developed since the 1940s (Pech et al., 1947; Pech and Henry, 1947). These techniques are increasingly being improved in order to obtain rapid and grouped germination (Corrrado et al., 1990; Gasselin-Durand et al., 2000). The technique of "germination by dry heat" developed by the Institut de Recherches sur les Huiles Oléagineuses (IRHO, Côte d'Ivoire) induces grouped germination in six weeks with 70% of germination. This technique requires seeds storage for 6 months after harvest, at a temperature of 20 to 22°C and a relative humidity of 60%, followed by alternated soaking and heating for 4 months, before the germination of seeds at room temperature (Corrado and Wuidart, 1990). This process is very long (10 to 12 months) and expensive and is not quite effective in ensuring a sustainable supply of selected seedlings to farmers.

The aim of this work is to contribute to the improvement of the technique of "germination by heat dry" With regards to the storage duration. It would help to develop techniques to shorten the germination delay of seeds to satisfy the strong demand of oil palm seedling by producers. The effect of storage and heat treatment of the seeds on their ability to germinate is studied on two strains of oil palm.

MATERIAL AND METHODS

Plant material

The seeds of two lineages of *Elaeis guineensis* Jacq., variety Dura provided by the Centre National de Recherche Agronomique (CNRA) in Côte d'Ivoire (West Africa) were used for this study. The linear LM 19954 provides the seeds category C70XX and the linear LM 19 617 provides the seeds category C24XX. LM 19954 is the product of a cross of female parent Dura (DA DA3D x 115D) x (115D x DA3D DA) with an unknown male parent and LM 19 617 is from a cross of female parent Dura (LM404D) x (DA 115D) with a unknown male parent. These linears of oil palm are the products of a genetic selection program. For each genotype, three lots of 4000 seeds each were used for germination tests:

- Lot 1: fresh seeds no stored after harvest (0 month of storage);
- Lot 2: seeds stored for 3 months after harvest;
- Lot 3: seeds stored for 6 months after harvest.

Methods

Seed storage

At maturity, 5 to 6 months after their formation on the palm trees, seeds are harvested, fermented in water for one week, and pulped. Seeds were then washed, dried, and disinfected with a fungicide (Benlate 0.5 to1.0 g.l-1 + Dithane M45) in 10 l of water to prevent the formation of mould. The seeds of Lot 2 and Lot 3 were kept in a room at 21 ± 1°C and a relative humidity of 60% for respectively 3 and 6 months. The seeds from the Lot 1 were immediately subjected to germination test after the harvest.

Seed moisture content determination

The moisture content of seeds was determined before germination test. Twenty seeds of each batch were weighed in order to determine their fresh weight (FW) and then, dried in an oven at 105 °C for 24 h to obtain a constant dry weight (DW).

The humidity of the seeds is calculated by the following formula:

$$MC = \frac{FW - DW}{DW} \times 100$$

FW: fresh weight

DW: dry weight

Germination process

The technique of "germination by dry heat" comprises different steps of seed treatment (Corrado and Wuidard, 1990).

Seed soaking

Different lots of seeds were soaked separately in water, at room temperature, for 7 days. The soaking water was renewed daily. After soaking, seeds were treated with a fungicide for 10 to 15 min, to prevent the formation of mould as previously, described, and then spread on jute bags for their wiping.

Seeds heating

Seeds were placed in transparent polyethylene bags, tightly closed and then, transferred for heating in a room on wire mesh shelves which allow a full flow of hot air. The heating temperature is maintained at 39 ± 1°C. On the basis of the work of Corrado and Wuidart (1990), for determining the optimum heating time for seeds germination, the different batches of seeds were heated to 39 ± 1°C for 40, 60 and 80 days. After heating, seed were

Table 1. Combined effect of storage and heating length on seed germination of both genotypes (C24XX and C70XX)

Seed category	storage (months)	Percentage of germination (%)					
		Heating time (days)					
		40	60	80	Mean	F	P
C24XX	0	32.16 ± 18.98a	67.293 ±9.87a	54.68 ± 17.68a	51.37 ±9.48ab	1.24	0.30
	3	33.99 ± 7.66a	38.29 ± 3.89a	33.93 ± 8.10a	35.40 ±3.48ab	0.30	0.87
	6	33.05 ± 10.20a	38.50±14.16a	27.85 ± 6.82a	33.13 ± 5.62b	0.24	0.79
Mean		33.07 ± 6.58b	48.02 ±7.02ab	38.82 ± 7.18b		F = 0.07	P = 0.92
C70XX	0	49.19 ± 6.04a	66.29 ± 0.75a	63.73 ± 10.15a	59.40 ± 4.43a	2.05	0.21
	3	52.01 ± 6.75a	70.28 ± 3.70a	46.20 ± 11.06a	56.17 ±5.31ab	2.60	0.15
	6	33.74 ± 6.59a	55.54 ±12.79a	43.40 ± 12.58a	44.22 ±6.35ab	0.98	0.43
Mean		44.65 ± 4.26ab	64.04 ± 4.43a	51.11 ± 6.48ab		F = 0.07	P =0.92

Means followed by the same letter are not significantly different at = 5%; the values represent the average of cumulative percentage, after 8 weeks at 25-28°C of each seed category; lots of seeds (0, 3 and 6 month's storage) were first, heated at 39°C for different time (40, 60 and 80 days) during germination process.

soaked in water for 5 days (with soaking water renewed daily) and cleaned. Seeds were then placed in polyethylene bags at room temperature (25-27°C) for germination, during height weeks.

Evaluation of seed germination

Germination percentage of seeds was evaluated each week, during two months. Seed are considered germinated when the radicle emerged from the testa. The percentage of germination (%G) was equal to:

$$\%G = \frac{NSG}{NST} \times 100$$

NSG: number of seed germinated

NST: total number of seed tested

After 8 weeks, the cumulative percentage of germination was determined.

Statistical analysis

Data were processed using Statistica 6.0. Variance analysis (ANOVA) was applied in order to find a significant difference (at = 5%) between the different treatment of the seeds and seed moisture on the effi-ciency of seed germination.

RESULTS

Germination percentage

The germination of seeds observed after 8 weeks, showed a varied cumulative percentage, ranged from 27.85 to 70.28%, depending on the types of seeds, length of seeds heating and storage time. No significant difference was found for the percentage of germination of the two categories of seeds with the various treatments (Table 1). However, the highest germination percentage (70.28%) was obtained with seeds of the category C70XX, stored for 3 months and heated for 60 days, while the lowest germination percentage was observed with the seeds of genotype C24XX stored for 6 months and heated for 80 days. Considering each genotype of seeds, the highest percentage of germination (67.29%) of seed type C24XX was obtained with fresh seeds heated at 40°C for 60 days, while the lowest germination percentage (27.85%) was obtained with the seeds stored for 6 months and heated at 40°C for 80 days. Seed of genotype C70XX shows the highest germination percentage (70.28%) was obtained when stored for 3 months and heated for 60 days. When stored for 6 months and heated for 40 days presented the lowest percentage (33.74 %) of germination (Table 1).

Genotype effect

The two genotypes used for this study differ significantly for germination percentage. A global consideration of seeds germination showed a mean percentage of germination of seeds of category C70XX from LM 19954, significantly higher (53.26%) compared to the germination (39.97%) of seeds of category C24XX from linear LM 19617 (Table 2).

Effect of seed storage

There is a difference between the germination of fresh seeds, seeds stored for 3 and 6 months after harvest. The germination capacity of seeds, when all heating treatment are cumulated, decrease with the length of storage.

Table 2. Genotype effect on seed germination.

Descendant	Seed category	Percentage of germination (%)	
LM 19617	C24XX	39.97 ± 4.03 a	F = 6.55
LM 19954	C70XX	53.26 ± 3.27 b	P = 0.013

Means followed by the same letter are not significantly different at = 5%; the values represent the average of cumulative percentage (after 8 weeks) of two categories of seeds, combining the different lots of seeds (0, 3 and 6 month's storage) and the different duration of heat treatment (40, 60 and 80 days) at 39°C; seeds were kept to germinate at 25-28°C after germination process.

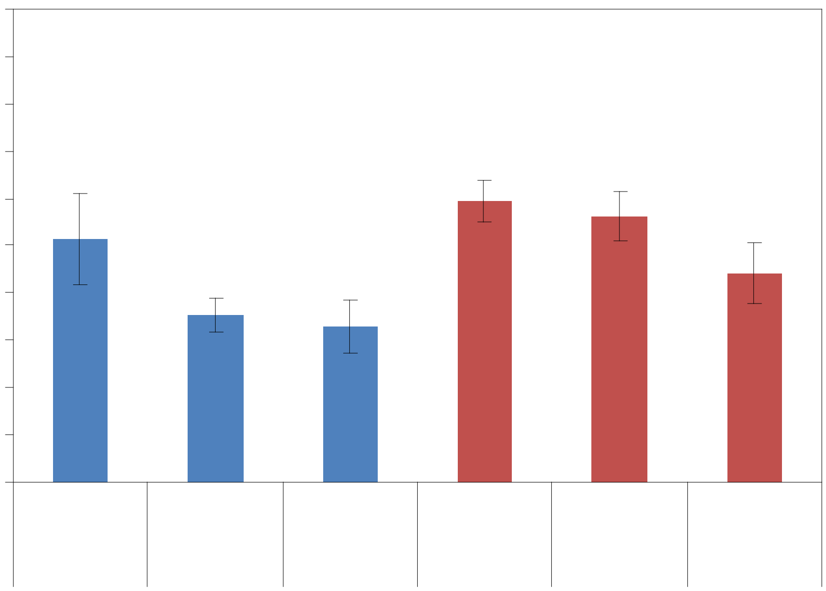

Figure 1. Effect of the length of storage and the genotypes on oil palm seed germination. V24 seeds category C24XX; V70: seeds category C70XX; GF: Lot 1 (fresh seeds; 3M: Lot 2: seeds stored for 3 months); 6M: Lot 3 (seeds stored for 6 months); values represent the average of cumulative percentage of germination (after 8 weeks) of seeds combining the different duration of heating (40, 60 and 80 days at 39°C); seeds were kept to germinate at 25-28°C after germination process; bars represents the standards errors.

For the two genotypes of seeds, fresh seeds (no stored) had the highest percentage of germination and the lowest percentage is obtained with seeds stored for 6 months (Figure 1).

Statistical analysis showed a significant effect of seeds storage on their germination rate. Seeds of Lot 1 (fresh seeds) resulted in significantly highest germination percentage (55.39 %) than those stored for 3 and 6 months (respectively 45.78 and 38.68%) (Table 3).

Table 3. Effect of seeds storage length on germination.

Storage time (months)	Percentage of germination (%)	
0	55.391 ± 5.17 a	F = 3.43
3	45.785 ± 3.98 ab	P = 0.039
6	38.681 ± 4.33 b	

Means followed by the same letter are not significantly different at = 5%; the values represent the average of cumulative percentage (after 8 weeks) of seeds stored for different time (0, 3 and 6 months) combining the two categories of seeds (C24XX and C70XX) and the different heat treatment (40, 60 and 80 days of heating at 3°C; seeds were kept to germinate at 25-28°C after germination process.

Effect of initial water content on seed germination

For the 2 types of seeds, storage allowed a significant decrease of water content (Table 4). Fresh seeds which gave the highest germination rate obviously contains the highest water rate and the lowest water content is shown with seeds stored for 6 months, thus the lowest germination rate.

Table 4. Water content of seeds stored for different periods.

Seeds category	Water content (% of dry weight)				
	Length of storage (months)			Statistics	
	0	3	6	F	P
C24XX	14.85 ± 0.06 b	10.38 ± 0.07 d	9.77 ± 0.09 e	324.29	0.000001
C70XX	16.01 ± 0.26 a	10.77 ± 0.11 c	8.85 ± 0.06 f	119.70	0.000015
Mean	15.33 ± 0.32 a	10.57 ± 0.19 b	9.31± 0.24 c	429.29	0.000001

Means followed by the same letter are not significantly different at = 5%; seeds water content were determined by heating about 20 seeds of each lots (0, 3 and 6 month's storage) at 105°C for 1 day to obtain a constant dry weight.

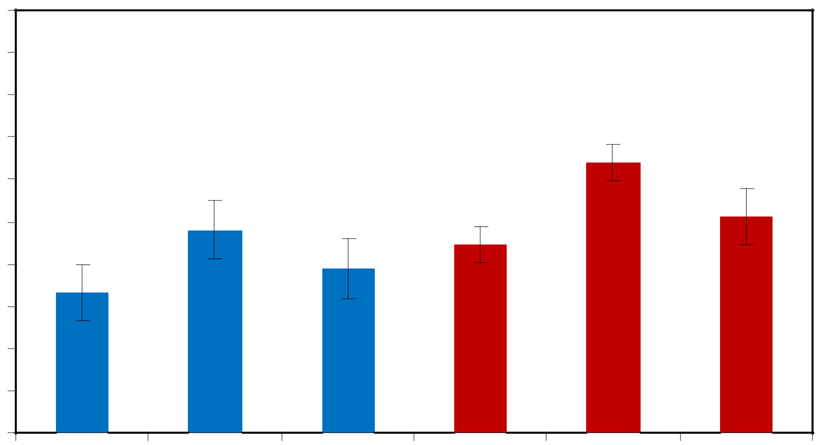

Figure 2. Effect of heating length and genotype on oil palm seed germination. V24/40-V24/60-V24/80: category of seeds C24XX, heating for 40, 60 and 80 days; V70/40-V70/60-V70/80: category of seeds C70XX, heating for 40, 60 and 80 days; values represent the mean of seeds germination cumulative percentage (after 8 weeks) combining to the different lots of seeds (stored for 0, 3 and 6 months); seeds were kept to germinate at 25-28°C after germination process; bars represents the standards errors.

Effect of heating treatment of the seeds

Whatever the genotype, the length of heating treatment has a significant effect on seed germination (Figure 2). For each genotype, seed heating at 40°C during 60 days allowed the best germination rate than the heating for 80 days. The heating for 40 days reduced strongly germination of seeds. This effect of heating treatment is significant when considered the average percentage of germination of both seeds category and storage length. Heating for 60 days leads to a better germination percentage of seeds (56.03%) than that of the heating during 80 days (44.96%) and 40 days (38.85%). The results of Table 5 indicate that these two latter heating treatments inhibit seeds germination.

DISCUSSION

The seeds of oil palm used in this study germinate independently to the genotype and the duration of seed storage period. Seeds require however, for germination, a heating at a temperature which varies from 38 to 40°C. Rees (1962) and Addae-Kagyah et al. (1998) also showed for several species of palms, the need of an exposure to relatively high temperatures of seeds to facilitate their germination. The capacity of seed germination however is strongly influenced by the genotype, the duration of the storage and heating.

The results showed a significant difference between the germination rates of the 2 seed genotypes used. The seeds used are from free fecundation of 2 linears (LM

Table 5. Effect of heating time on oil palm seed germination.

Length of heating days	Percentage of germination (%)	
40	38.85 ± 4.05 b	F=3.74
60	56.03 ±4.47 a	
80	44.96± 4.92 ab	P= 0.03

Means followed by the same letter are not significantly different at = 5%; the values represent the average of cumulative percentage (after 8 weeks) of seeds heated for different time (40, 60 and 80 days at 39°C) combining the two categories of seeds (C24XX and C70XX) and the different duration of storage (0, 3 and 6 months); seeds were kept to germinate at 25-28°C after germination process.

19954 and LM 19617) of palm trees. So, only female genotypes of the parents are known. Seeds of the category C70XX showed a better global germination rate when submitted to germination process than those of the category C24XX. This first category of seeds can be used for the genetic selection program for seedlings production to the farmers. Koebernik (1971) and Wagner (1982) also found percentage of germination which differs from specie to another and even within specie. Clement and Dudley (1995) also demonstrated different germination capacities of seeds of different cultivars of *Bactis gasipaes* in the answer to environmental conditions.

Fresh seeds showed the highest percentage of germination if we consider the average of germination rate of each genotype of seeds for combined heating and storage duration treatments. The germination capacity of seeds decreased with the duration of their storage.

The study highlighted a global significant effect of the length of storage on oil palm seed germination rate. But, storage effect varies, however, when genotype and heating duration of seeds are considered. Seeds of certain palm such as *Metraxylon warburgii* and *M. vitiense* germinate rapidly after their fall of the tree (Doren, 1977). While others, such as *Elaesis guineensis, Plagodoxa heuryana*, can germinate only after several years (Braun, 1984; Phillips, 1996). This long period of germination could be due to embryo immaturity at the time of the seed fall (Phillips, 1996; Yang et al., 2007) or to teguments impermeability of water and oxygen uptake. This impermeability is gradually eliminated with the storage duration or by mechanical and chemical treatments (Robertson and Small, 1977; Baskin and Baskin, 2000) . According to our results, non-stored seeds are able to germinate with a significantly higher germination rate than seeds stored for different periods. So, this type of dormancy may not be present in the two genotypes of oil palm used for the present study. Thus, it is not necessary to store oil palm seeds for 6 months, before starting the germination process. The technique of "germination by dry heat" developed by Corrado and Wuidart (1990) could then be improved by the use of fresh seeds which would allow the shortening of oil palm seedling production of 6 months.

The physical and physiological initial events in the seed appear to play a predominant role in germination. Indeed, according to Berjak et al. (1984) the ability of the seed to respond positively to external stimuli for germination depends on its initial conditions at the time of application of the stimuli. The fresh seeds have relatively higher water content than that of seeds stored for 3 and 6 months. The high percentage of germination of the seeds appears to be related to their high initial moisture content as shown by Martins et al. (2003) with king palm seeds. However, in details, the effect of seed initial water content may be associated to heating treatment. It seems that, seeds stored for 3 months have the best response for a heating period of 40 days, while for a heating period of 80 days, fresh seeds gave the best response to germination. For the heating duration of 60 days both fresh and 3 month's stored seed reacted positively to the germination process. In any case, the seeds stored during 6 months seem to have a non optimum initial condition for an efficient germination.

The positive effect of heat on seeds germination has already been demonstrated by several authors (Nagao et al., 1980; Moussa et al., 1998; Addae- Kagyah et al., 1988; Herrero et al. 2007). Particularly for oil palm, Rees (1962) showed that heat treatment is essential to improve seed germination. The technique used for oil palm seed germination was initiated by Rees (1963) and improved by Corrado and Wuidart (1990). This technique consists on heating seeds at 40°C for 80 days. Our results showed, however, that the best heating period, which gives a germination percentage around 70 % is 60 days. Heating for 80 days significantly reduces the germination of the seeds, while 40 days of heating appears insufficient to induce optimal seed germination. Germination tests realised with seeds heated for 20 days shown no germination (data not shown). The heating of seeds combined with their soaking, activates the cellular metabolism of the embryo, which results in its germination (Robertson and Small, 1977, Moussa et al., 1998; Oracz, 2007). Improving oil palm seeds germination by the heat treatment, allowed classifying them in the group of exogenous dormancy (Cirak et al., 2007). It is important to note that the optimum heating temperatures are around 40°C (Rees, 1962). The heating time after soaking, is thus a key factor in improving the germination of oil palm seeds.

Conclusion

The results of this study indicate that fresh seeds (none stored) of E. guineensis Jacq. Var. Dura, have a higher germination percentage than seeds stored for 3 and 6 months. That could allow reduction of the production time of seedling for 6 months with a better result. The heating duration of 60 days is the optimal time to improve the percentage of seed germination. Oil palm seeds germination relies strongly on the genotypes of different lineages developed. These different conditions for obtaining seeds germinated within a short period could provide significant solutions to the numerous demands of oil palm producers.

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