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Effect of dry heat treatment along with some dormancy breaking chemicals on oil palm seed germination

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1. Introduction

The African oil palm (*Elaeis guineensis* Jacq.) is by far the most productive oil crop and plays an important role in fulfilling the large and growing global demand for vegetable oils, estimated to reach 240 million tons by 2050 (Corley, 2009). The production of palm oil has been increasing at a rapid rate due to the overwhelming global demand for edible oils and oil products, oleo-chemicals and for clean transportation energy fuel. The lengthy germination period of oil palm seeds presents a major drawback for the production and supply of seedlings at a commercial level (Tabi et al., 2016). Two main constraints naturally affect the propagation of oil palm. Firstly, the oil palm is one of the rare members of the Arecaceae family that do not produce suckers and the only natural method of propagation is via seeds. Secondly, germination of oil palm seeds is naturally very slow, low and unsynchronized (Hussey, 1958; Hartley, 1988). Considering that in practice the propagation of oil palm is accomplished mainly by seeds, such difficulties in germinating seeds have serious consequences at the agronomic and socioeconomic scales. The low germination of oil palm seeds has been attributed to physical dormancy (Hussey, 1958) and this is a major constraint in producing planting materials (Rees, 1962). The early works on oil palm germination was carried out by Hussey (1958) who attributed the slow and low germination in oil palm to the endocarp's hard and dense consistency which gives mechanical strength to resist the absorption of oxygen. In a recent study, Norsazwan et al. (2016) reported that oil palm seeds have a combination of both morphological and physical dormancies. In yet another study, Kaewtaphan et al. (2016) postulated that oil palm seed dormancy may be classified as a complex dormancy of mechanical restriction and morphophysiological dormancy. However, the fact that the stony endocarp alongside the layers of hard oily endosperm prevents the uptake of water as reported by Moussa et al. (1998) cannot be underestimated. Even though the endocarp has been reported to be permeable to external factors like water and oxygen (Robertson and Small, 1977), the physical barriers may appear to delay these external factors from reaching the thresholds needed to initiate growth in the embryo (Hussey, 1958).

Hussey (1958) was the first to shed light on the use of a high temperature (38–40 °C) treatment to overcome dormancy in oil palm seeds. This was followed by Rees (1962) who reported that a minimum moisture content of 17% is necessary for seeds subjected to dry heat treatment for 80 days in order to optimize germination. Other authors (Addae-Kagyah et al., 1988; Corrado and Wuidart, 1990; Herrera et al., 1998; Fondom et al., 2010; Martine et al., 2011; Green et al., 2013) have carried out studies aimed at improving the protocol laid down by Hussey (1958) and Rees (1962). It is probably for this reason that dry heat scarification treatment (DHT) has remained the conventional technique in oil palm seed production centres around the world. In spite of several studies aimed at ameliorating the DHT protocol, germination rates continue to be slow, low and unsynchronized. Probably this unsatisfactory germination rate is because the DHT alone does not completely eliminate other internal factors that hinder the embryo from emerging. Therefore this study was carried out with the hypothesis that germination stimulating chemicals when integrated with the DHT will play a summative effect to rupture physical, morphological and physiological dormancy thereby improving germination percentage. The aim of the study was therefore to measure the synergistic effect of DHT and some growth promoting chemicals on germination of oil palm seeds.

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2. Materials and methods

2.1. Study site

This study was carried out in the Oil Palm Seed Production Unit of the Specialized Centre for Oil Palm Research, Institute of Agricultural Research for Development, (IRAD-CEREPAH) of La Dibamba, located at 3.948848° N, 9.762726° E and 55 m above sea level in the Littoral Region of Cameroon.

2.2. Plant materials

Seven cultivars of *tenera*-type oil palm (*Elaeis guineensis* Jacq.) seeds were used in this study. They were obtained through controlled pollination of *dura* (D) × *pisifera* (P) parent palms of the CEREPAH breeding program. The codes used to designate the different tenera hybrid samples and their parents (D × P) are, C1901II (LM 19171 × LM 18978), C2001II (LM 17164 × LM 18978), C2101II (LM 17163 × LM 18978), C2501II (LM 17685 × LM 18978), C1001IIF (LM 19016 × LM 19029), C2301IIF (LM 18744 × LM 19029), and C2501IIFX (LM 13533 × LM 19029).

Six months after controlled pollination, fresh fruit bunches were harvested, stripped, allowed to ferment and depulped. Immature and cracked seeds were eliminated by hand sorting. Seeds were then treated with 2.5 g/L contact fungicide (Penncozeb®) solution for 5 min. Seed samples were then air dried and stored in an air conditioned room at 20–25 °C and 60% relative humidity. All seeds used in this experiment had been under such storage conditions for 4–5 months.

2.3. Growth promoting chemicals

Three plant growth promoting chemicals (GPCs) supplements used in this study were 30% w/w (110) extra pure hydrogen peroxide (H_2O_2) obtained from Scharlab S.L. Spain, 90% pure gibberellic acid (GA_3) and 50 wt% cyanamide solution (CH_2N_2) both manufactured by Sigma-Aldrich, Germany.

2.4. Preparation of stock solutions of GPCs

For GA_3, three stock solutions of different concentrations 0.5, 1.0 and 1.5 g/L (denoted as G1, G2 and G3) respectively were prepared by dissolving the respective amounts of 90% pure GA_3 powder in 10 mL of 96% ethanol for 10 min followed by filling with portable water to 1 L. The solution was stored between 25 and 30 °C. Initial addition of GA in alcohol was to facilitate dissolution given that GA_3 dissolution is very slow in water at ambient temperature. Three different concentrations denoted H1, H2 and H3 for 0.5%, 1% and 1.5% respectively were prepared from the original solution. For hydrogen cyanamide (CH_2N_2), three stock solutions denoted C1, C2 and C3 corresponding to 1, 1.5 and 2% were prepared from an original solution of 50 wt% commercial formula.

2.5. Dry heat scarification and treatment of seeds with GPCs

The DHT applied in overcoming oil palm seed dormancy in this study was a synchronized modification of the different steps of seed treatment to initiate germination as proposed by Hussey (1958), Rees (1962), Addae-Kagyah et al. (1988) and Corrado and Wuidart (1990). A sample of 250 oil palm seeds from the same fresh fruit bunch was randomly collected to constitute the sample for each oil palm cultivar. The seeds were weighed, put in water bags permeable and soaked in a water bath (filled with portable water) for 7 days at ambient temperature in order to raise the water content to 18%. The water was renewed daily to minimize the growth of fungi. Prior to daily re-immersion, the seeds were weighed in order to determine the rate of imbibitions. On the 7th day, the seeds were treated with a contact fungicide (Penncozeb®) for 2–3 min and air dried under shade. The fungicide solution was prepared by dissolving 4 g of the solute in 10 L of portable water. Thereafter the seeds were put in transparent polyethylene bags and positioned on wooden shelves in a heating room at a constant temperature of 40 °C. The heating room was furnished with temperature adjustable heaters and ceiling fans to ensure an even distribution of hot air in all parts of the room. After 80 days at 40 °C, seeds were removed from the heating room and subjected to the different treatments.

For the control (DHT only), samples of 20 seeds per cultivar were selected randomly and re-soaked in water for 5 days in order to raise their moisture content to 22%. This was followed by soaking for 2–3 min in a solution of Penncozeb® (4 g in 10 L of water). Samples were then air dried under shade, packaged in transparent polyethylene bags and allowed to germinate under ambient conditions. Until the seeds start showing visible signs of germination, packaged seeds were misted with water once a week. Germination parameters were evaluated after two weeks for the first assessments and monthly over the later period of 120 days.

A random sub sample of 20 seeds per cultivar that had been exposed to the DHT (40 °C) for 80 days was weighed and re-soaked in 100 mL of the respective GA_3 test solutions for 48 h. Further soaking was done in portable water for 3 days (renewed daily) so as to raise the moisture content to 22%. Likewise for H_2O_2, randomly selected sub samples of 20 seeds per cultivar was re-soaked in 200 mL of corresponding concentrations of 0.5%, 1% and 1.5% solution of H_2O_2 for 48 h and thereafter renewed daily for 3 days (renewed daily) with successive measurement of MC until 22%. The similar method was used for CH_2N_2, where random sub sample of 20 seeds per cultivar were taken following DHT and re-soaked in the respective concentrations of 1, 1.5 and 2% CH_2N_2 for 2 days and then in water for 3 days (renewed daily). After treatments with GPCs, samples were dried under shade and placed in strong transparent polyethylene bags and kept on wooden shelves under ambient conditions. As with the control, seeds were misted once a week until signs of germination were visible. Germination parameters were assessed on a weekly basis for 8 weeks. All seeds that did not germinate were subjected to the 2, 3, 5 triphenyltetrazolium chloride (TTZ, 0.1%) test to check their viability.

2.6. Germination parameters and method of assessment

Of the several parameters used to study the dynamics of the germination process (Ranal and Santana, 2006), germination capacity (final germination), time and rate were chosen in this study. Germination capacity (GP) refers to the total number of seeds germinated in a seeds lot and it is calculated as:

$$GP = \frac{\text{Number of germinated seeds}}{\text{Numberoftestedseeds}} \times 100$$

For germination time, the mean length of incubation time (MLIT) was preferred to the median time or time to 50% germination due to the asymmetric nature of germination frequency. MLIT is a measurement of the mean duration required for maximum germination of a seed lot, and is expressed in terms of the same units of time used in making germination counts (hours or days). It was calculated as:

$$MLIT = \frac{(G1T1 + G2T2 + \ldots + GnTn)}{(G1 + G2 + \ldots + Gn)}$$

where G: germination count on any counting period; and T: time (Czabator, 1962).

Germination rate represented as coefficient of velocity of germination (CVG) gives an indication of the rapidity of germination. CVG increases when the number of germinated seeds increases and the