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Seed germination and ultra structural changes in oil palm (*Elaeis guineensis*) hybrid seed influenced by heat treatments

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ABSTRACT

Seed dormancy in oil palm (*Elaeis guineensis* Jacq.) is considered as one of the major causes for low and erratic germination. Oil palm hybrid seeds (*dura* × *pisifera*) were subjected to heat treatment for 0, 10, 20, 30, 40, 50, 60, 70, 80 and 90 days in a heating room at 39 + 1°C and germination response, ultrastructural changes in embryo, endosperm and operculum structures were observed. The results revealed that seed heating for 50, 60 and 70 days and incubation in germination room (25 to 27 ºC) resulted in germination of 90.4, 93.6 and 94.8%, respectively. Heating of seeds for 0, 10 and 20 days had no effect on germination. Structural changes of dormant and germinating seeds were investigated through microtome sectioning and Scanning Electron Microscope (SEM). Endosperm above the embryo is demarcated by several layers of small cells. During the break of seed dormancy, endosperm cleaves in the micropylar region through the small cells. Enlargement of embryo facilitates the dislocation of the operculum during the germination. It is confirmed that heat treatment for 60 to 70 days to be optimum for obtaining maximum oil palm seed germination. Nevertheless, heating oil palm seeds at 50°C is recommended for maximum germination in a short time.

Key words: Germination, Heating durations, Oil palm, Seed dormancy, Ultra structures

Oil palm (*Elaeis guineensis* Jacq.) is a perennial tropical tree which is being propagated predominantly by seeds. Seed dormancy is considered as a major cause for low and erratic germination which affects quality planting material production in oil palm (Martine *et al.* 2009). It is necessary to break the dormancy, usually through heat-treatment (Green *et al.* 2013). Although, many researchers have been trying various approaches to shorten dormancy period, heat treatment is only still adopted for commercial purpose. There are several reports of effect of heat treatment and genotype influence in oil palm seed germination response (Fondom *et al.* 2010 and Green *et al.* 2013). Hoyle *et al.* (2008) advocated that the period during which seeds develop on the parent plant has been found to affect seed dormancy through interaction with the environment. Seeds from different genotypes, latitudes and even ripeness may shows differences (Beugré *et al.* 2009).

Physical characteristics of seeds of different genotypes of oil palm have been studied in order to understand germination behavior (Myint *et al.* 2010a and Myint *et al*. 2010b). Some enzymatic activities in germinating oil palm seeds has been studied by Oo and Stumpf (1983). Scanning Electron Microscopy observations is useful to know the detailed information concerning surface changes during germination and seed development (Mariani *et al*. 1999) and ultra structural studies concerning the seed and the zygotic embryo provide important information for improving the seed germination (Baskin and Baskin 2004). Although oil palm seed germination was reported by many authors, detailed investigation on the mechanism underlying the rupture of the barrier structures has not been reported. Hence, the effect of heat treatment duration on germination and changes in the embryo, endosperm and operculum structures has been investigated.

MATERIALS AND METHODS

The fourteen years old *dura* mother palm was hybridized with *pisifera* in the seed garden. The hybridized bunch was harvested after 165 days of fruit maturity and kept for three days for loosening of fruits. The mesocarp of fruit was removed by mechanical de-pulper and seeds were extracted. The extracted seeds were washed with detergent to remove remnants of oil and individual seeds were scrapped with knife to ensure fibre attached with seeds and mesocarp remnants removed. Seeds were surface dried under shade until the adhering moisture evaporated and packed in lots of hundred seeds into 20×40 cm size 500 gauge polythene bags. The open end was tightly secured with packing rope after trapping enough air inside the bag. The bags were immediately kept in racks installed in an electrically operated

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heating room maintained at 39 + 1 °C for up to 90 days. Treated seeds were drawn at intervals of 10 days. Seeds which did not receive heating treatment were considered as control. Each treatment was replicated three times and the seed lots of 100 seeds were included in each replication. All the heat treated seeds were soaked in water for five days and after shade drying for two hours adjusted for moisture content of 22% and put back in the new polythene bags and incubated at 25 to 27 ºC in a germination room. Observation on days required for initiating germination, total number of days to reach maximum germination, cumulative germination percentage were recorded at four days intervals up to 80 days. Seed germination during first flush was recorded between 18 and 22 days as described by Mok and Hor (1977). As 0, 10 and 20 days heat treated seeds did not germinate, data from 30 to 90 days (7 treatments) heat treated seeds were alone taken for statistical analysis. In the histological study, two days after incubation in germination room, five seeds from each treatments were collected kernels extracted from the seeds in each treatment were given 'V' shaped cutting and the embryo was dissected with adjoining endosperm without disturbing the operculum (plate like structure present in the seed coat above the embryo). The embryo adjoining endosperm below the operculum was sectioned using microtome (Leica model RM 2125) and investigated for changes in the seed structures. Seed coat, endosperm and zygotic embryo of samples of seeds were fixed for 48 hr in FAE50 (formalin: acetic acid: 50% ethyl alcohol, 5:5:90, v/v/v) and stored in 70% ethanol were transversally sectioned in a table microtome as described by Johansen (1940). The sections were stained with Toluidine blue (O'Brien *et al*. 1965) mounted with DPX and photographed under a light microscope. For SEM study, seeds were dried in desiccators before mounting on aluminum stubs. Each seed was sputter coated with gold for six minutes before viewing in a Scanning Electronic Microscope at an accelerating potential of 6 KV with a magnification of 50×500 ìm. Ultra structural changes in the seed coat structures around the operculum were recorded for the representative samples of heat treated and control seeds.

RESULTS AND DISCUSSION

Effect of heat treatment on germination

The results indicated that heat treatment duration significantly affected the oil palm seed germination. There was no germination in seeds that received zero and 20 days of heating durations. Similar result of germination percentage was reported in heat treatments of 0, 10 and 20 days by Alizaga *et al.* (2012). In heat treated seeds, the first flush germination occurs usually with 6-10 days of lag period, which will last for 10 days (Mok and Hor 1977). The germination differences were recorded in commercial oil palm seeds due to the effect of heat treatment at 39 + 1°C for different durations (Mok and Hor 1977). The initiation of seed germination required 30 days of heat treatment and the maximum germination percentage (94.8%) was recorded in 70 days of heat treatment within 18 -22 days (first flush). Wonkyi-Appiah (1974) also observed that *dura* seeds, which received 70 days heat treatment, resulted in the maximum germination than 80 days heat treatment. The heat treatment period for 50 days also resulted in satisfactory germination (90.4%). Similar results were observed by Addae-Kagyah *et al.* (1988) in *idolatrica* oil palm under Ghana climatic conditions. Jimenez *et al*. (2008) reported that seed producing companies attempted 50 days heat treatment to break seed dormancy in Costa Rica. In the case of initiation of germination, heating periods of 50, 60, 70 and 80 days took lesser time, viz. 29, 22, 18 and 22 days after incubation, respectively. Seeds pre-heated for 40 days took 60 days for maximum germination. Greater water imbibitions during the initial stages of heat treated seeds during incubation under germination room were reported by Neves *et al.* (2013). It was interesting to note that the seeds heated for 60 and 80 days, took maximum germination of 93.60 and

Table 1 Capacity and rate of germination of *dura* seeds at different duration of heat treatments under ambient room temperature.

Heating duration (days)	Days to initial germination	Days to maximum germination	Flush germination *(%)	Final germination (%)
0				
10				
20				
30	7	79	7.50	32.00
40	4	76	32.00	64.40
50	9	29	88.00	90.40
60	6	22	93.00	93.60
70	6	18	94.80	94.80
80	6	22	53.00	53.70
90	8	32	61.70	62.80
CD (0.005)	1.51	1.42	2.17	4.93
SE(d)	0.71	0.67	1.03	2.30
CV (%)	19.04	2.96	2.93	5.80

*The percentage of seed, which germinated within first 18-22 days after incubation in germination room.

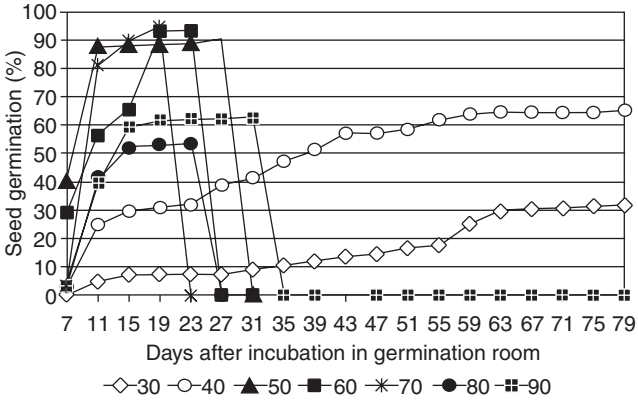

Fig 1 Cumulative germination of seed germination affected by different duration of heat treatment.

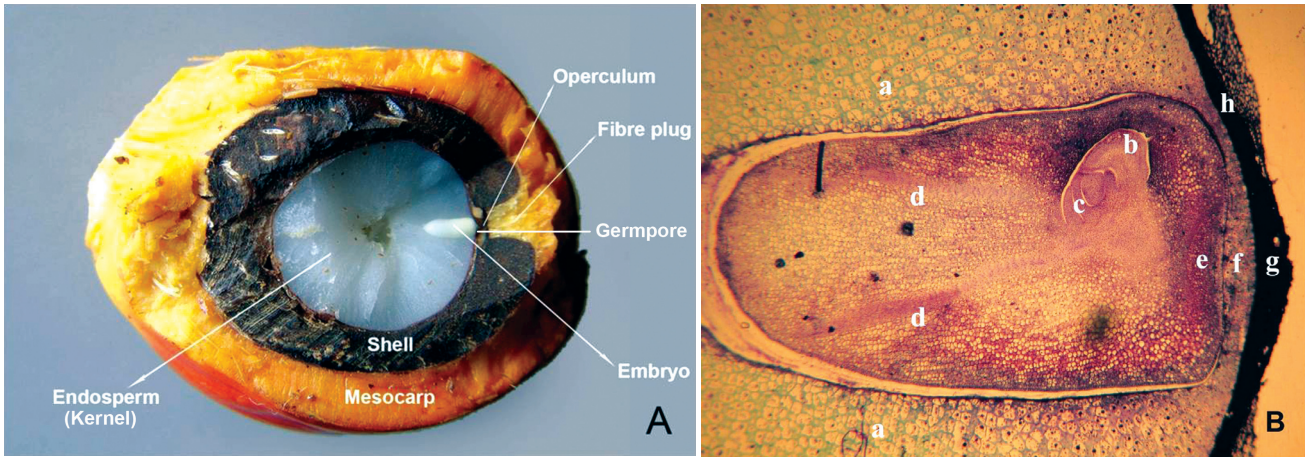

Fig 2 Oil palm seed and embryo structure (A and B). A : Longitudinal section of oil palm fruit indicating the germpore filled by fibre plug below that operculum and embryo (micropylar endosperm + seed coat) under the germination pore. B. Longitudinal microtome section of dormant seed embryo and endosperm (a. Endosperm, b. Radicle, c. Plumule, d. Haustorium's band, e. Quiescent cells, f. Micropylar region, g. Operculum extension of seed coat, h. Seed coat).

53.70%, respectively within 22 days. Increased heating period beyond 70 days resulted in sharp decline in germination percentage (53.7%) in 80 days and 62.8% in 90 days heating periods. Seeds exposed to high temperatures beyond 70 days might have prevented translocation of food materials from the endosperm to the embryo (Table 1).

Faster germination may be a consequence of higher seed vigour (Association of Official Seed Analysis 2002, Tekrony and Egli 1991) or the result of a partially broken dormancy or combination of both factors. The above findings confirm that heat treatment for 60 and 70 days followed by five days soaking of *dura* seeds to be optimum to get maximum germination (>94%). Nevertheless, heating oil palm seeds at 50ºC is also recommended for maximum germination in a short time (Fig 1).

Anatomical changes in embryo, endosperm and operculum

Morphological structure of oil palm seed was described and diagrammatically illustrated (Hussey 1958). In palm seeds, the endosperm cell wall is generally thicker because of the deposition of cell wall storage polysaccharides; mainly the hemicellulose like mannan (Buckeridge *et al.* 2000). The germpore present in the nut is blocked by a plug of fibres which are cemented at the base to form a plate like structure called 'operculum'. The seed (kernel) coat or testa is thicker where it covers the distal part of the embryo. There is transition region between the lateral and micropylar endosperm with small cell volumes and cell walls. Micropylar endosperm volume is less than the remaining endosperm. The plate like structure 'operculum' is an extension of the seed coat. Longitudinal section of oil palm seed (A) and microtome section of dormant seed embryo (B) given in the Fig 2. Morphological differences exist between the cells of the micropylar and lateral endosperm (Williams *et al.* 2001, da Silva *et al.* 2005). During the germination, the emerging embryo forms a button of tissue which quickly develops a plumule (shoot) and a radicle (root) and at the same time, the other end of the embryo enlarges to form a cotyledonary structure called the haustorium (Cheang Oo 1983). In the present study, a ring of cells of small size demarcates the endosperm above the embryo which forms micropylar endosperm (Fig 3A).

In oil palm seeds embryo growth is principally restricted by the rigid cell walls (rich in mannans in *Phoenix dactylifera* a member of *palmae*) of the micropylar endosperm (Gong *et al.* 2005, Moura *et al.* 2010). Seed germination begins with

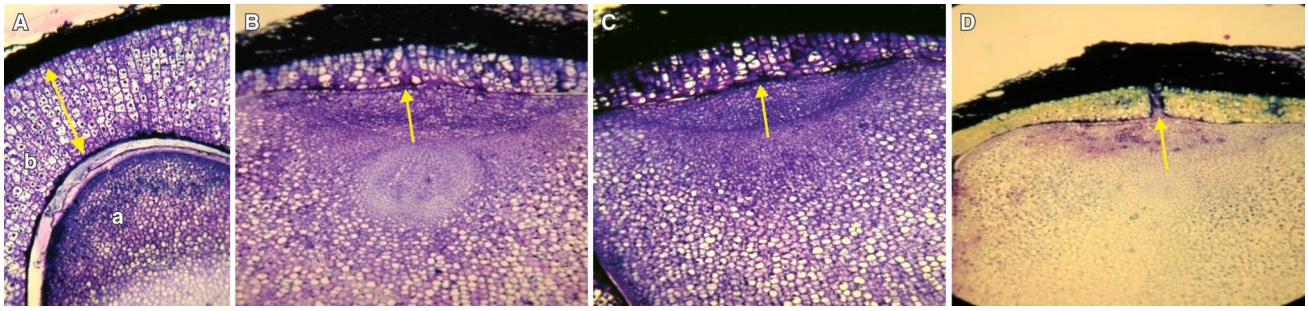

Fig 3 Histological changes during germination after thermal heated with different durations A. (0 D heating) Normal seed a. Endosperm cell wall is thicker than embryo cell wall (see the arrow), Ring of small cells which demarcates embryo and micropylar endosperm B,C & D Heat treated seeds. B and C Embryo exterting pressure to distal endosperm, embryo enlarge in distal centre and growth pushing micropylar endosperm D. Cleave (indicated with arrow) formed by enlarging embryo in the micropylar endosperm.

water uptake and ends with the emergence of the radicle or plumule through the surrounding seed tissues (Bewley *et al.* 2013) and is a consequence of the break of the mechanical force of surrounding tissues by the growth of embryo (Nambara *et al.* 2010). During germination of oil palm seeds, rupture of the operculum appears to be is dependent upon intercellular breakdown in the abscission layer (of the micropylar endosperm) as well as pressure exerted by the growth of embryo (Hussey 1958). Structural alterations of the endosperm tissue of treated seeds were observed at the micropylar area of the region of radicle protrusion (Alang *et al*. 1982). Heat treatment weakens the abscission layer, which borders the operculum and reduces the force necessary for the embryo to rupture this layer. Jiménez *et al.* (2008) reported reduction of abscisic acid concentration after the heat treatment of oil palm seeds. In the present study, embryo pushing through the thin walled cells of endosperm is shown in Fig 3 B, C and D whereas, no structural changes was observed in dormant seeds (Fig 3A). In advanced stage, embryo (Fig 3 D) has penetrated entire wall of micropylar endosperm. To break through the testa and eventually produce a radicle and plumule, the embryo must grow about 1.5 to 3.0 times their initial length (Perez *et al.* 2008). Continued embryo growth before radicle protrusion has been documented for palm such as spineless 0sago palm (Ehara *et al.* 1998). Completion of germination is marked by the radicle emerging through the testa and underlying endosperm with the operculum splitting away from the remaining testa along the furrow. The weakening of abscission layer is initiated by the hemicellulase, endo ß mannanase (Bewley 1997). In oil palm seeds, the walls of the endosperm cells in the micropylar region are weakened by hydrolytic enzymes or the cell wall structure and or composition of the endosperm in the micropylar region is modified becomes less resistant to radicle penetration (Hussey 1958). During germination, the embryo forces its way out through the germpore. Hence, it is accepted that weakening of the endosperm particularly in the micropylar region adjacent to the radicle is a prerequisite for the completion of germination. The slightest elongation of the embryo will cause rupture of the operculum and once this has taken place germination continues unhindered (Baskin and Baskin 2014). Yu Zhang *et al.* (2014) reported that activities of enzymes do not increase prior to radicle protrusion, indicating that they are unlikely to play important roles. It is still unclear which enzymes are responsible for cell wall loosening in radicle protrusion and endosperm weakening in seeds. Hence, much remains to be learned about germination of palm seeds, both at the whole-seed and biochemical–molecular levels (Baskin and Baskin 2004). SEM study revealed that operculum centre of seed coat had lignified cells and prominent wax like coat in the untreated seed confirmed that in dormant seed operculum is firm and act as a barrier to water entry to the underneath structures like micropylar endosperm and embryo. When embryo grows during the germination, it displaces the operculum and afterwards the radicle protrudes. Before the displacement, cracks developed in the operculum.

It is concluded that heat treatment for 60 to 70 days to be optimum for maximum oil palm seed germination. Nevertheless, heating oil palm seeds at 50^{o}C is recommended for maximum germination in a short time. There is a transition region between the lateral and micropylar endosperm in the seed (kernel) and critical changes that occur in the junction between growing embryo and micropylar endosperm facilitates the dislocation of the operculum during the seed germination. In dormant seeds operculum became depressed in the centre point, whereas treated seeds exhibit cracks.

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