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Aseptic Storage of Elaeis guineensis form pisifera Seeds

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There is a gene in the African oil palm (*Elaeis guineensis*) that controls the type of fruit produced. In the homozygous state one allele yields the *dura* form with a thin shell (stony endocarp) and relatively little pulp (mesocarp). In the homozygous state the other allele yields the *pisifera* form with a substantial amount of pulp but no shell. A *dura* × *pisifera* cross gives rise to the heterozygote or intermediate form —the *tenera*. This form has a thin shell, produces a substantial amount of pulp and comprises the oil palm of commerce (see Hartley, 1977 and references there cited). *Pisifera* palms are of importance not only because they yield oil but because they are used as the pollen parent in breeding programs (Obasola, 1973).

Unfortunately *pisiferas* are frequently female-sterile and their seeds usually have a much reduced level of germination. Moreover, the lack of a stony endocarp (cf. Fig. 1A) renders the seeds very prone to desiccation and microbial contamination. This problem is so extreme that much of the difficulty encountered in the germination or storage of the seeds derives from it. A relatively simple technique which permits an increased level of germination has recently been described (Nwankwo, 1981). The method involves aseptically de-operculating the seed so as to expose the germinal end of the embryo and in this way facilitate germination. Even so, all published work on *pisifera* seed germination has thus far involved the use of freshly harvested seeds, because of the lack of an effective means of storage. This has restricted seed studies to that relatively limited period of bunch availability (e.g. in Nigeria ripe bunches are more abundant during the dry season—November to March) and to the geographical area of production.

In this report we describe a reliable method for prolonging the life of *pisifera* seeds. The procedure can also greatly facilitate inter-continental transport and germplasm exchange with regions outside the area of normal availability. It may well be that seeds of other palms which present difficulties of microbial contamination or have limited viability could be handled in a similar way and for this reason we wish to draw special attention to it.

Procedures

Preparation of Seeds. Mature seeds (=kernels comprised of the testa, endosperm and embryo) obtained from open-pollinated bunches at the Nigerian Institute for Oil Palm Research were extracted from fruits by cutting the mesocarp with a sharp knife and ejecting the contents. Up to this point ordinary laboratory or field conditions are satisfactory. When the required number of kernels are obtained they are taken to a "clean room" where they are surface-sterilized and prepared for shipment. The seeds are first submerged for 5 minutes in a 0.2% aqueous

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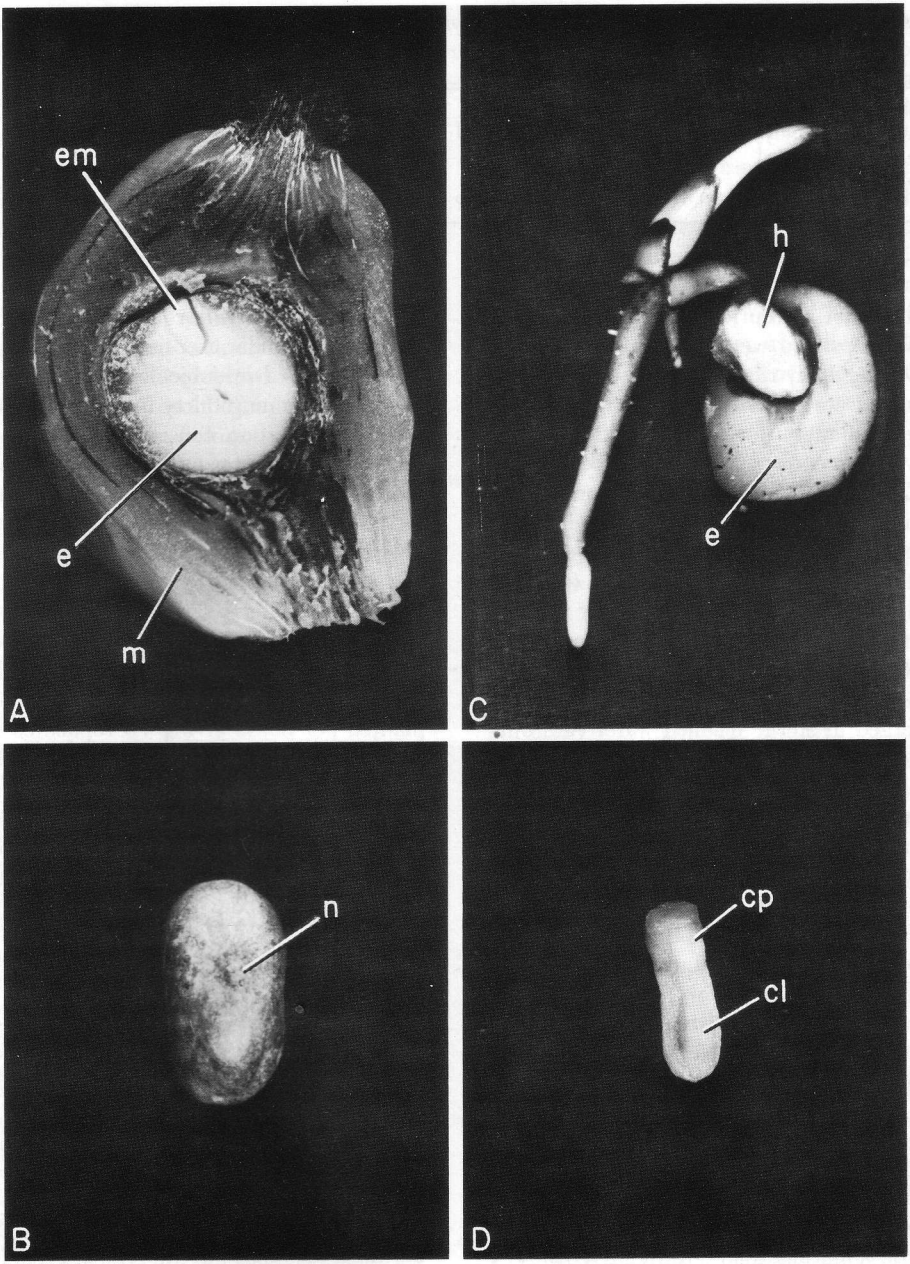

1. *Pisifera* oil palm fruit, seed, embryo and seedling. A. Fruit in vertical median section showing fleshy mesocarp (m), endosperm (e) and embryo (em). The endocarp is comprised of fibrous tissue but lacks the stony components found in the *tenera* and *dura* forms. (Magnified 2.1×). B. External appearance of a *pisifera* kernel or seed. The line points to the "notch area" (n) from which the germinal end of the embryo would emerge during germination. (Magnified 2.3×). C. Aseptically stored and germinated seed cut so as to expose the haustorium (h) and endosperm (e). (Magnified 1.94×). D. Embryo excised from *pisifera* kernel such as that shown at B. The germinal end or petiole limb or cotyledonary petiole (cp) is separated by a slight constriction from the cotyledonary limb (cl) or haustorial end. (Magnified 8.3×).

Table 1. Effect of the length of storage on the appearance and percent germination of pisifera *seeds stored aseptically in water for 15, 30, and 60 days*

Storage (in days)	15	30	60
External appearance of seeds	Normal	Normal	Normal
No. of seeds planted	30	30	30
No. of seeds infected	0	0	0
% Germination	90.0 ± 7.5	82.0 ± 6.5	68.4 ± 7.2

15 replicates (2 seeds each bottle).

solution of mercuric chloride containing a few drops of a wetting agent (e.g. *Tween 20*) and then rinsed 3 times with sterile distilled water. The seeds are then submerged again for 5 minutes in a 5% (v/v) aqueous solution of Chlorox (i.e. 0.264% v/v sodium hypochlorite) and rinsed 3 times in sterile distilled water. The seeds are then transferred aseptically into plastic screw-cap containers (ca. 500 ml) which can be pre-sterilized by treating with mercuric chloride solution and then exhaustively rinsed with sterile water. After the seeds have been completely submerged in sterile distilled water (use only enough to cover the seeds), the bottles can be tightly twisted shut and the lids sealed with parafilm strips to keep the seals clean. The bottles can then be packed into a plywood box or other suitable container and transported by air. This has been repeated 3 times between Murtala Mohammed Airport, Lagos, and John F. Kennedy Airport, New York. An interval of up to ten days between seed processing and its receipt in New York has been recorded.

Re-sterilizing Seeds upon Receipt. Upon receipt, the bottles were wiped with 70% ethyl alcohol, opened and the water decanted in a clean room. The contents were re-sterilized first using 0.2% mercuric chloride followed by a 10% v/v aqueous solution of Chlorox (i.e. 0.525% v/v sodium hypochlorite). The seeds were again covered with sterile distilled water, the bottles tightly sealed and stored in a growth chamber at 30 ± 1° C in darkness.

Viability Tests. Samples of 40 seeds each were taken after 15, 30, and 60 days storage under aseptic conditions. Ten were employed in subjective examination and the remaining thirty in viability tests. Visual observation consisted of examination of the seeds for any abnormality that may have developed during storage and for microbial contamination. This was followed by embryo excision from the seed and visual examination of the embryo (cf. Fig. 1D). The thirty seeds for viability tests were de-operculated by cutting the notch from which the embryo would normally emerge (cf. Fig. 1C). Two seeds were then planted per bottle giving 15 replicates on sterile foam rubber moistened with sterile distilled water and placed in darkness at 30 ± 1° C. Emergence of the plumule and radicle from the embryonic axis was taken as evidence of germination (see Fig. 1B for an embryo about 18 days from "planting").

Results

After the first fifteen days of storage the aseptically treated seeds remained fresh and no microbial contamination could be observed. The untreated controls (i.e. those which had not been re-sterilized upon arrival had become contaminated. Embryos excised from re-sterilized seeds were turgid and looked healthy with the germinal end showing the typical greenish yellow coloration (see Fig. 1D for an excised embryo, unfortunately it is not in color). Up to 90 percent germination (see Table 1) was recorded after 15 days storage out of thirty seeds planted. These gave rise to healthy seedlings (see Fig. 1B).

Viability tests conducted after 30 days storage gave similar results. There was no contamination. The greenish coloration of the embryos appeared even more conspicuous and 82.0 ± 6.5% germination was recorded. Tests carried out after sixty days storage gave similar results but with a slight reduction in response. There was no contamination. The embryos looked healthy and a germination record of 68.4 ± 7.2 percent was obtained. Embryos of seeds which did not germinate were mostly white or brownish and probably died as a result of intrinsic abnormalities such as failure in haustorium development etc. and not due to contamination.

Discussion

No effective means that we are aware of has hitherto been described for the storage of *pisifera* seeds. Until recently germination has had to be carried out promptly after harvesting. Even in Nigeria investigations involving *pisifera* seeds have been hampered during that time of year when *pisifera* supply is limited. The intrinsic value of a simple method such as the one described lies in the ability to store seeds under aseptic conditions, thus making them available at all seasons and facilitating equally successfully their transportation to distant places. The relatively high germination response of *pisifera* obtained even after 60 days storage derives from the elimination of microbial contamination from the germinating seeds. No doubt the length of storage could be extended beyond 60 days but the level of viability is likely to diminish.[1] However, even *dura* and *tenera* seeds which are protected by a shell and hence are not seriously affected by micro-organisms during storage are not completely trouble-free. These seeds can be and are stored at controlled temperatures (usually at about 21° C and about 18–20% moisture content) without surface sterilization for over 12 months. But losses as high as 40% have been reported due to the so-called "brown germ" disease wherein the embryo darkens and dies. This is especially prevalent when seeds are germinated under conditions of high moisture and temperature (i.e. the "wet heat treatment", Aderungboye, 1977). Using the technique of storage reported here *pisifera* seeds can be maintained in a moist environment thus precluding desiccation and permitting their vigorous germination on "planting" in aseptic culture.

[1] Over 50 percent germination has been reported from *pisifera* seeds stored in unaerated sterile distilled water for six months (Nwanko and Krikorian, 1982).

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