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Seed Characteristics of African Accessions of Oil Palm (*Elaeis guineensis*)

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ABSTRACT

The germination percentage of shell-less *pisifera* and thin-shelled *tenera* seeds is extremely low. Recently oil palm seeds were collected in Tanzania, Senegal, Sierra Leone, Gambia, and Guinea to broaden the genetic base. The *pisifera* seeds were germinated in vitro while the *tenera seeds* were germinated using both in vitro and in vivo methods. Current study shows that it was possible to establish more accessions from Gambia, Guinea, and Senegal using the in vitro method. In the case of the Sierra Leone collections, more accessions were established by the in vivo method.

The Malaysian Agricultural and Development Institute (MARDI) initiated its first oil palm (*Elaeis guineensis* Jacq.) germplasm collection in 1973 (Rajanaidu and Rao 1987). Since 1979 these responsibilities have been carried out by the Palm Oil Research Institute of Malaysia (PORIM). These collections are essential to the oil palm industry to broaden the genetic base of current breeding materials, which had eroded through years of extensive breeding and selections (Rajanaidu 1985*a*, 1987) and also for germplasm conservation (Hardon 1985). Now, PORIM has the world's largest oil palm germplasm repository involving about 400 ha of land planted with 60,000 seedlings from Africa and Latin America. The oil palm plantations will benefit from these collections when new genes are incorporated into existing breeding materials (Rosenquist et al. 1988, Rajanaidu and Rao 1987). Other oil palm genotypes with desirable traits such as short trunk or high unsaturation oil were also collected (Rajanaidu 1985*b*, Rajanaidu et al. 1989). Some exotic palm species such as *Bactris gasipaes* (Pejibaye), *Oenocarpus* spp. and others with potential economic value were also included in the collections (Rajanaidu et al. 1991).

A review of practical applications of embryo culture technique was made by Raghavan (1977). One of the applications is the culture of zygotic embryos to rescue them from hybrid genetic crosses (Mott 1984, Dunwell 1986). Yuri (1987) used embryo culture to propagate wine palm, which normally germinates poorly by the conventional method. This technique has also been applied to overcome poor germination of *pisifera* seeds and stored seeds (Rohani and Paranjothy 1985, Paranjothy et al. 1989). Sterile conditions have also been used to store *pisifera* kernels; germination (%) after 60 days of storage was still high (Nwankwo and Kirkorian 1983). The embryo culture method involves removing the embryos from the surrounding endosperm tissue and culturing them on agar nutrient media supplemented with growth substances either in the absence (Jones and Dethan 1973) or presence (Rohani and Paranjothy 1985) of activated charcoal, or on a basal nutrient medium devoid of growth substances but solidified with Gelrite (Paranjothy et al. 1989). Embryo rescue technique is now being used routinely to germinate seed germplasms in vitro. This method was also used to germinate mantled fruits in vitro to study the meiotic transmission of abnormal characteristics in oil palm (Paranjothy et al. 1993).

In mid 1993 and early 1994, PORIM officers carried out an expedition to collect *tenera* and *pisifera* germplasm in Africa. A major proportion of the germplasm seeds was germinated in vivo using the method described by Hartley (1988) and about 20 seeds per accession were subjected to embryo culture. This paper describes the preparation of the African seed materials before and after they were dispatched to Malaysia, germination using in vitro and in vivo methods, and seed characteristics. Comparison between the two methods for

establishing plant collections from seed is discussed.

Materials and Methods

Seed Collection. Tenera fruits were collected from Gambia, Guinea, Senegal, and Sierra Leone. Approximately 64,000 seeds were collected from 266 accessions. The fruits were depericarped at the collecting centers to remove the nuts. The nuts were washed, dried, and packed before they were transported to the International Mycological Institute, Surrey, London, UK, for quarantine. After one to three months the seeds were dispatched to the Malaysian Phytosanitary Department (MPD) for further quarantine and inspection against pests and diseases (Rajanaidu 1994). Only 'clean' seed consignments were released to PORIM. Of 266 accessions, 260 were finally released by the Phytosanitary Department. The Senegal and Gambian seeds were prospected in July 1993 and Guinean and Sierra Leone in April/May 1994. The embryos were cultured in September 1994, 14 months after collection of the Senegal and Gambian seed, and five months after collection of the Guinean and Sierra Leone seed.

Shell-less *pisifera* fruits were collected in Sierra Leone and Tanzania in May and July 1994, respectively. Since they could not be stored for long periods special arrangement had been made to dispatch this fruit form to Malaysia in the shortest time possible. *Pisifera* fruits were manually dehusked and the kernels obtained were treated with fungicide. They were dispatched to the MPD for further inspection before they were released to PORIM. Embryo culture was carried out after two weeks of storage.

Seed Germination by the Embryo Culture Method. The basal medium was made up of MS (Murashige and Skoog 1962) macro- and micronutrients, Y3 vitamins (Eeuwens 1976), 0.1 g/L inositol, 0.1 g/L glutamine, 0.1 g/L arginine, 0.1 g/L asparagine, and 3% sucrose. The medium was solidified by adding 0.15% Gelrite. The pH of the medium was adjusted to 5.7. Ten ml of medium were dispensed into 25 × 150 ml tubes. The media were autoclaved at 121°C for 15 minutes.

Seedlings attaining a shoot height of ≥3 cm were transferred to 10 ml of rooting medium containing 6% sucrose, 5–9 × 10^{-5} M α-naphthalene-acetic acid, and 0.15% activated charcoal.

The cultures were incubated in the light room with a photoperiod of 12 hours and the room temperature was maintained at 28° ± 1°C (Paranjothy et al. 1989).

Kernel Preparation. Tenera seeds were placed in clear plastic bags and the shells were broken with a hammer. The kernels were picked out with a pair of clean forceps and placed in 250-ml conical flasks, one accession per flask. Partially broken kernels were also utilized as long as the operculum remained intact and the embryo was not damaged or exposed. On reaching PORIM (10 days after collection), *pisifera* seeds were further dehusked and the kernel surfaces were scraped clean with a scalpel blade. The kernels were washed several times with distilled water before transferring them to petri dishes lined with filter paper to absorb excess water and were then air-dried overnight. The drying was necessary because the residual oil in the mesocarp tissues and the exposed oily endosperm surfaces made the kernels slippery and difficult to hold during embryo excision.

Surface Sterilization. All sterile manipulations were carried out in the laminar air flow cabinets. The surgical instruments, glassware, water and 4 × 8 cm polypropylene sheets were sterilized before use. The sterile petri dishes (25 × 150 mm) were all lined with two layers of sterile filter paper.

The kernels were transferred to sterile conical flasks and immersed in water completely. The flasks were shaken manually for one to two minutes before decanting the water into another container for disposal. The washing was repeated seven times and 0.05% Tween 20 was added to the last washing. This was followed by washing twice with 0.1% of mercuric chloride ($HgCl_2$) and 0.05% Tween 20 solution for 5 minutes each. The kernels were then rinsed with water five times at two minute intervals, followed by soaking in water for 10 minutes with occasional shaking. The above rinsing steps were repeated. After decanting the water, the kernels were transferred to sterile petri dishes.

Embryo Cultures. The opercular membrane was flipped open with the tip of a blade to expose the embryo base. The germ end was turned downwards and with a soft tapping on the kernel with a scalpel or spatula the embryo slipped free onto the polypropylene sheet. Sometimes, a small excision on the endospermic tissues was needed to help free the embryo. The embryos were lifted individually with a warm, sterile spatula or a pair of forceps and transferred to a culture tubes. Only uninfected and undamaged embryos were used. After eight weeks of culture, the number of embryos

Table 1. Establishment of seedlings derived from in vitro *and* in vivo *germination methods.*

			In vitro				*In vivo*			
No.	Germplasm regions	Seed+ type	No. of seeds/ accession	Total no. embryos cultured	Total no. established	% established	No. of seeds/ accession	Total no. sown	No. germinated	% germinated
1.	Tanzania	P	19–125	81	9	11.1	—	—	—	—
2.	Sierra Leone	P	60	34	7	20.6	—	—	—	—
3.	Gambia	T	17–21	644	301	46.7	10–390*	14415	14	0.1
4.	Guinea	T	8–11	574	177	30.8	77–286	13183	974	7.4
5.	Senegal	T	19-40**	1788	836	46.8	130–442	27178	586	2.2
6.	Sierra Leone	T	9–11	497	93	18.7	87–362	9319	1572	16.9
	Total			3618	1407	38.9		60095	3146	5.2

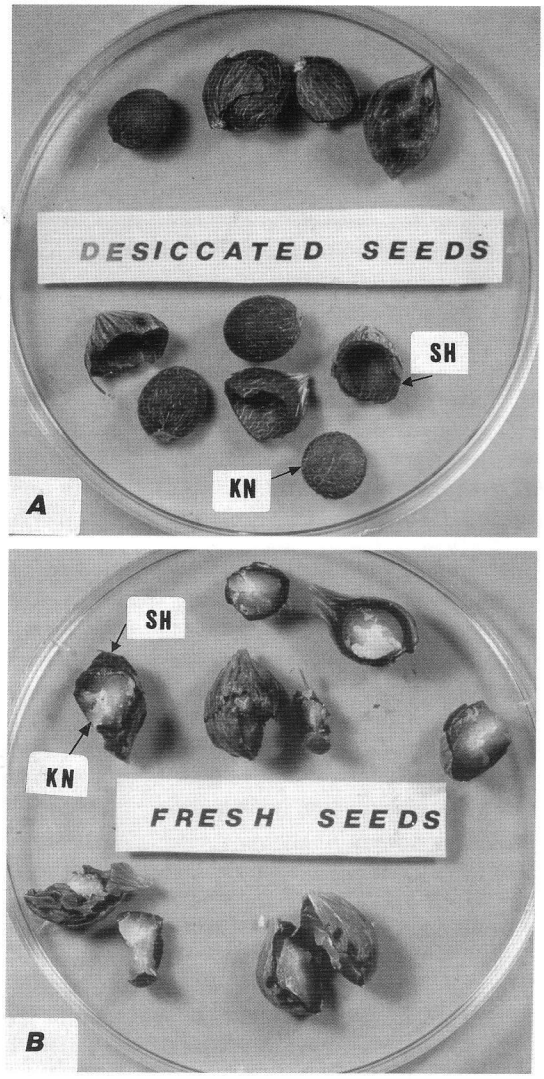
+ P = *pisifera*, T = *tenera*.
* Eight accessions with ≤30 seeds.
** One accession with 40 seeds, others 19–21 seeds per accession.

developing into a shoot or both a shoot and a root was determined as the germination success.

Establishment of Seedlings in Soil. Seedlings with well-developed root systems were transferred to 10 × 17.5 cm perforated polybags containing sand and soil mixtures (1:1). They were hardened in outside enclosures with 80% shade and watered daily (Fig. 1).

Seed Germination by the Conventional Method. Conventional seed germination was carried out at PORIM Research Station, Kluang. The seeds were incubated in the heating chamber at 40°C for 60 days followed by soaking the seeds in water for 4–5 days. After soaking, the seeds were placed in plastic bags and incubated at the ambient temperature for 10 days. The number of seeds producing both plumule and radical were scored to determine germination percentage.

1. Four-month-old germplasm seedlings in prenursery.

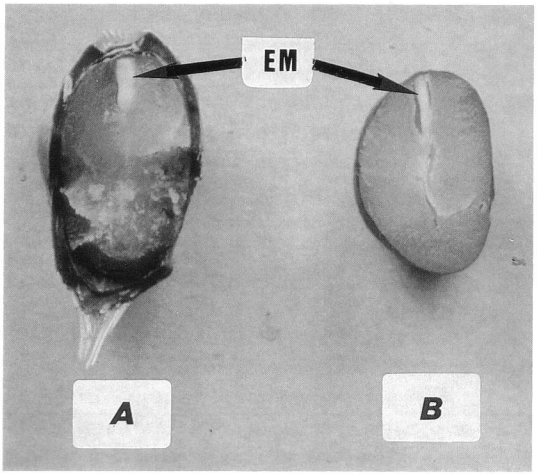


2. (A) Desiccated seeds—the shells (SH) broken to release whole kernels (KN). (B) Fresh seeds—the shells (SH) broken together with the kernels (KN).

Disposal of Materials. Shell remnants, infected seeds and embryos, and those damaged from severe desiccation were autoclaved for 45 minutes before they were discarded.

Observations and Discussion

Tenera seeds were received, fully dehusked with shells intact. About 60,000 germplasm seeds were germinated in vivo and approximately 3,600 seeds were germinated using the in vitro method (Table 1). Stored seeds were normally slightly desiccated and it was easier to break the shells compared to fresh seeds (Fig. 2). It was also observed that embryos in desiccated seeds shrank slightly (Fig. 3), and this made it easier for them to slide through the opercular opening by tapping the kernel gently with a spatula. Severely desiccated embryos were very brittle and broke easily; thus, they were never cultured.



3. Longitudinal sections of (A) fresh seed and (B) stored seed (desiccated). The embryos (EM) in the desiccated seed shrank slightly.

Shell-less *pisifera* seeds are more vulnerable to desiccation and to bacterial and fungal infections (Nwankwo and Krikorian 1983, Hartley 1988). Therefore, they are seldom stored, but are cultured soon after they are harvested.

Seed Characteristics. Pisifera seeds are known to have limited viability (Paranjothy et al. 1989) and this contributes to poor germination in vivo compared to *duras* or *teneras.* A large number of the seeds are parthenocarpic (embryo-less). Table 2 shows that parthenocarpy occurred in $\geq$33% of *pisifera* seeds and in $<$2% of *tenera* seeds. Previous study showed that the percentage parthenocarpy of 10 P $\times$ P crosses varied from 4 to 97% (Rohani and Paranjothy 1985). This study also showed that a high fertility rate did not always guarantee a high rate of germination. Besides parthenocarpy, seed dormancy could also cause poor germination.

Another characteristic observed was multiple kernels, i.e., having more than one kernel per seed. In these collections the percentage of multiple kernels in *tenera* seeds ranged from 2.8 to 4.9 (Table 2). Not all accessions had multiple ker-

Table 2. Observation on seeds of different fruit forms from different germplasm regions.

No.	Germplasm regions	Seed* type	Total seed examined	Multiple kernels		Parthenocarpic		Infected	
				No.	%	No.	%	No.	%
1.	Tanzania	P	324	0	0	219	67.6	7	2.2
2.	Sierra Leone	P	60	0	0	20	33.3	4	6.7
3.	Gambia	T	716	35	4.9	12	1.7	96	13.4
4.	Guinea	T	600	22	3.7	3	0.5	44	7.3
5.	Senegal	T	2027	79	3.9	26	1.3	291	14.4
6.	Sierra Leone	T	539	15	2.8	2	0.4	57	10.6
	Total		4266	151	3.5	282	6.6	499	11.7

* P = *pisifera*; T = *tenera*.

nels. Table 3 shows that about 36% (highest) of Guinean accessions have multiple kernels and 13% (lowest) in Sierra Leone's. At least one Sierra Leone accession and three from Senegal had multiple kernels in >41% of the fruits. Three Gambian and two Guinean accessions had multiple kernels in 21–40% of the fruits. Generally <20% of fruits had multiple kernels per accession. It is common to obtain multiple kernels in some seed types such as *duras* or *teneras* (Corley and Gray 1976, Hartley 1988). Multiple kernels in *pisifera* have not been observed to date, although a number of P × P seeds were examined in the past.

Comparison Between in Vitro and in Vivo Germination Techniques. Compared to conventional seed germination techniques, the embryo excision and rescue technique is both labor intensive and requires special skills. The conventional method can handle several thousand seeds at a cheaper cost and seeds germinate in a short time. Seedlings derived from embryo cultures require rooting, transplanting, and hardening in a prenursery, all of which takes about seven months, while in vivo germination requires about three months.

In dealing with valuable genetic seeds, it is of utmost importance to ensure the survival of some of the accessions. Since germplasm seeds have undergone prolonged storage during transit, it is impossible to predict their success rate. There are several factors that could affect the germination success of the seeds in vivo. These are moisture content, temperature (Hussey 1958, 1959, Hartley 1988), and storage duration. Thus, germination by other methods may be necessary to serve as a back-up in case of poor germination using the conventional germination method. This is well demonstrated in Gambian seeds (Table 1) where only 0.1% of a total of 10415 seeds using in vivo methods established successfully compared to a higher rate using the embryo rescue technique (46.7% of a total of 644 seeds).

The establishment of seedlings from each accession in the collection is crucial. Each accession represents seed materials obtained from palms growing in different climatic and soil conditions. Table 4 indicates that the embryo rescue method produced a higher (%) of successful accessions than the conventional method with the exception of Sierra Leone. Although the germination of seed embryos of Sierra Leone in vitro and in vivo was identical (17–19%, Table 1), the in vivo method was better for salvaging accessions (93%) than the in vitro method (65%, Table 4). Embryos that did not germinate in vitro either pro-

Table 3. Tenera *accessions with multiple kernels from various germplasm regions.*

No.	Germplasm regions	No. of accessions					
		Total	With multiple kernels	%	Fruits with multiple kernels		
					≤20%	21–40%	≥41%
1.	Gambia	60	11	18	8	3	0
2.	Guinea	36	13	36	11	2	0
3.	Senegal	100	29	29	26	0	3
4.	Sierra Leone	54	7	13	6	0	1

Table 4. Establishment of tenera *accessions by* in vitro *and* in vivo *methods.*

No.	Germplasm regions	*In vitro*			*In vivo*		
		Total no. of accessions	Successful accessions		Total no. of accessions	No. of Successful accessions	
			No.	%		No.	%
1.	Gambia	36	35	97	44**	3	7
2.	Guinea	60	52	87	60	44	73
3.	Senegal	102*	98	98	102	47	46
4.	Sierra Leone	54	35	65	54	50	93
	Total	252	220	87	260	144	55

* Two accessions were 100% contaminated and therefore were not cultured.
** The number of seeds from eight accessions were ≤30, therefore they were not supplied to the tissue culture laboratory.

duced haustoria only, which later became necrotic, or remained dormant. The dormant embryos did not show any evidence of infection even after keeping the cultures for about eight weeks.

Conclusions

Of the two methods used to germinate stored germplasm seeds, the embryo rescue technique, in general, maintains a higher percentage of successful accessions than the in vivo method. The in vitro technique is also useful to germinate shell-less *pisifera* seeds, which normally germinate poorly in vivo. The in vitro method does not require a large number of samples but does require specially trained skills. Because all the seeds are examined before embryo excision the in vitro technique can identify some causes of poor germination in vivo, such as parthenocarpy, bacterial and fungal infection, and damaged embryos. The success of the in vitro method demonstrates the value of this technique as a complementary approach to in vivo germination for the establishment of oil palm germplasm collections.

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