



## Differential pollen longevity in Dura and Pisifera oil palm (*Elaeis guineensis*) fruit types at storage temperatures

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Received: 7 October 2016; Accepted: 29 December 2016

### ABSTRACT

Oil palm (*Elaeis guineensis* Jacq.) is the richest source of vegetable oil in the world. Specific reproductive nature of the crop necessitates artificial pollination by collection and storage of pollen for breeding as well as generation of planting material for commercial cultivation. However, little information is available on the pollen viability and storage behavior of oil palm. The present study was conducted to standardize the processing and storage of oil palm pollen in respect of Dura and Pisifera fruit forms/types. Pollen of these two fruit forms were stored up to one year at room temperature (33±2°C), refrigerator (5°C), freezer (-5°C) and in deep freezer (-20°C) and the viability as well as germination were compared. For both the fruit types, pollen viability and germination declined as the storage temperature increased up to room temperature and the maximum germination was recorded in pollen stored under -20°C storage. Therefore, deep freezer can be used without any deleterious effect on pollen viability as well as germination. Pollen longevity was significantly reduced when stored at room temperature. Of the two oil palm fruit-types, Pisifera retained greater pollen viability and germination than Dura under all four storage temperatures. To the best of our knowledge, this is the first report of differential response of pollen from Dura and Pisifera fruit types in oil palm to storage temperatures.

**Key words:** Dura, Oil palm, Pollen, Pollen storage, Pisifera

The oil palm, a perennial oil yielding crop, is the richest source of vegetable oil in the world and can produce 5 MT of vegetable oil per ha per year. Cultivated oil palm is Tenera, a hybrid between thick shelled Dura (female parent) and shell less Pisifera (male parent). The hybrid nature of the crop necessitates artificial hybridization between proven palms which in turn require the pollen from male parent to be collected and stored without loss of viability. The ability of pollen to be stored for sufficiently long period is essential for breeding programmes, genetic conservation and artificial pollination (Lora *et al.* 2006). Longevity of the pollen is the period of time over which the pollen retains its viability, i.e. germinability and fertilization ability. A successful hybridization greatly depends on the quality of pollen, viz. viability and germination capacity. The pollen should retain sufficient viability and vigour for effective pollination and fruit set and thereby ultimate yield. During the storage period, factors such as temperatures and relative

humidity (RH) have effect on pollen longevity.

Pollen viability may decrease quickly depending upon the storage conditions. Long-term storage of pollen has been demonstrated for many plant species. Low temperature is an important condition, but the temperature recommended depends on the species (Lee *et al.* 1985, Hanna and Towill 1995). Pollen of three olive cultivars showed little loss in germinability over storage period up to one year at -20°C in controlled relative humidity (Pinney and Polito 1990). However, pollen from species like pistachio were very difficult to store in refrigerator or freezer as the viability is lost within a week (Vaknin and Eisikowitch 2000).

Dura and Pisifera are distinct fruit forms in oil palm (Hartely 1988) and hence pollen is expected to have variation in response to storage conditions. With respect to female inflorescence, Pisifera produces mostly sterile or partially fertile fruits clearly indicating the deviation in fertility status as compared to Dura which produces perfectly fertile flowers. However, there is no information available on storage behavior of pollen from these two oil palm categories/types. Storage of oil palm pollen in general was achieved in liquid nitrogen (Tandon *et al.* 2007). Myint *et al.* (2012) found differential response of Dura, Pisifera and Tenera for pollen germination. Variation in pollen quality among two different cultivars of date palm was reported by Mortazavi (2003) and Mortazavi *et al.* (2010), where they could store the pollen for up to 200 days in deep freezer.

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Dutta *et al.* (2013) found that excepting cryo storage, the highest viability of mango pollen was retained at  $-20^{\circ}\text{C}$  followed by  $-5^{\circ}\text{C}$  up to 24 weeks of storage. Oil palm pollen in general could be stored for 6-8 weeks at room temperature with silica gel or calcium chloride (Corely and Tinker 2003).

The optimum conditions for storage need to be defined especially when maintaining the pollen in LN is not feasible. This is especially important in case of germplasm sites and seed gardens which are often situated in far flung areas which restricts infrastructure for cryo-storage. Pollen viability in many genera may decrease quickly depending on storage conditions (Albuquerque *et al.* 2007.). Appropriate and efficient methods of pollen storage must be identified. This paper reports the effect of different storage temperatures on retention of longevity and germination of oil palm pollen in Dura and Pisifera fruit types.

#### MATERIALS AND METHODS

The study was conducted using pollen from two oil palm fruit types Dura and Pisifera palms of 15 years age, collected from Dura mother block and Pisifera (T×T) block grown at the Directorate of Oil Palm Research, Pedavegi, Andhra Pradesh, India. Pollen from different palms of same category was bulked to minimize the palm to palm variation.

The male inflorescence selected for pollen collection was bagged five to seven days before anthesis. The male inflorescences were isolated *in situ* with a pollen collection bag, of 74 cm × 86 cm size, having a celluloid or plastic observation window of 16 cm × 16 cm at the front. During the bagging process, the stalk at the bottom of the bag was wrapped around with a layer of rolled cotton wool dusted with insecticide then secured with a strong fine wire to prevent any contamination.

Male inflorescence was harvested together with the bag after 80-90 % of the flowers have anthesized. The remaining flowers were expected to open during the drying process in the laboratory. The bag was left to air-dry the moisture. The inflorescences still contained in the bag were then dried for about twenty four hours in the heat-room at  $38^{\circ}\text{C}$ - $40^{\circ}\text{C}$  for preliminary drying of the pollen grains. Upon completion, the bags were well shaken to dislodge the pollen grains from the spikelets. When all the pollen grains have been detached, they were transferred to paper bag and sieved using 150  $\mu\text{m}$  brass-sieve and contaminant-free pollen grains were poured into an envelope. Pollen envelopes were then put in oven at  $38$ - $40^{\circ}\text{C}$  for a second 24-hour drying to ensure that they were totally dry. Drying temperature was maintained at not more than  $40^{\circ}\text{C}$  to avoid damage to the pollen grains and the moisture was brought below 7%. While testing for *in vitro* germination it was rehydrated by spreading on clean cavity slide and keeping the slide in a petridish with moist filter paper (RH>95%) for one hour at ambient temperature.

The viability of the pollen was determined by 2,5-diphenyl tetrazolium bromide (MTT) (Sunilkumar *et al.* 2011). This detects the presence of dehydrogenase. The

test solution consisted of 1% of the substrate 2,5-diphenyl tetrazolium bromide (MTT or Thiazolyl blue) in 5% Sucrose. The viable pollen turned deep pink to violet and whereas dead pollen did not stain.

Pollen from the oil palm was cultured for *in vitro* germination using by sitting drop culture using suitable media (Tandon *et al.* 2001). The cultures were maintained in a humidity chamber to prevent evaporation. The cultures were kept for incubation in the humidity chamber under  $33\pm 2^{\circ}\text{C}$  temperature for two hours for fresh pollen and 12-24 hr for stored pollen.

To identify favorable storage conditions for oil palm pollen of Dura and Pisifera, the collected pollen samples were placed in 5 ml cryo vials with screw caps. The vials were subjected to storage under the following temperature regimes: room temperature ( $33\pm 2^{\circ}\text{C}$ ), refrigerator ( $5^{\circ}\text{C}$ ), freezer ( $-5^{\circ}\text{C}$ ) and deep freezer ( $-20^{\circ}\text{C}$ ). After every 30 days each sample was subjected to viability and germination tests up to one year of storage. The data was subjected to statistical analysis using SPSS statistics Version 21.

#### RESULTS AND DISCUSSION

Longevity is the ability of pollen to remain viable, germinate as well as to fertilize receptive ovule under congenial conditions. Results of the viability and germination tests of pollen from Dura and Pisifera are presented in Table 1 and 2 respectively and discussed separately.

*Longevity of Dura pollen:* Results of the data on viability and germination of Dura pollen is presented in Table 1.

Under room temperature ( $33\pm 2^{\circ}\text{C}$ ), there was significant reduction in viability after one month of storage, but the viability was retained above the minimum 60 % required to consider it as viable for all practical purposes. However, after two months of storage, the viability was drastically reduced rendering it unfit for pollination purposes. The viability after two months was on par with at third month after storage. The stored pollen showed no viability at four months after storage at room temperature. The germination declined drastically during second month and from third month onwards there was no germination at all, indicating that at room temperature, Dura pollen cannot be stored for more than a month. Kwon *et al.* (2005) reported that when water melon pollen was stored at  $-10$ ,  $4$  and  $10^{\circ}\text{C}$  temperatures, germination was greater at  $-10^{\circ}\text{C}$ .

In case of pollen stored at  $5^{\circ}\text{C}$ , viability after one month was on par with that of fresh sample. Then it declined significantly but was close to the 60% criteria for considering it as viable. At three months after storage, viability declined significantly and was insufficient to consider as viable. Pollen viability at four and five months after storage were on par but below the required levels. After 10 months storage no viable pollen was observed in the case of storage at  $5^{\circ}\text{C}$ . Germination percentages of pollen at  $5^{\circ}\text{C}$  temperature was in agreement with that of viability. Up to two months of storage, there was sufficient germination but it was significantly reduced and reached zero after 10 months storage. Akonda *et al.* (2012) observed significant difference in germination

Table 1 viability and germination of Dura pollen under different temperature regimes

Period of storage	Viability (%)				Germination (%)			
	33±2°C	5°C	-5°C	-20°C	33±2°C	5°C	-5°C	-20°C
Fresh	93.57	93.57	93.57	93.57	91.07	91.07	91.07	91.07
1 MAS	70.07	84.83	88.10	94.23	38.43	65.13	70.67	85.20
2 MAS	8.43	54.07	70.50	76.13	0.00	51.80	79.83	84.90
3 MAS	6.93	36.43	62.77	79.60	0.00	36.37	62.30	77.63
4 MAS	0.00	25.33	52.67	72.20	0.00	14.10	47.23	74.57
5 MAS	0.00	24.23	36.77	77.50	0.00	23.20	32.40	75.27
6 MAS	0.00	22.67	36.73	76.70	0.00	23.97	31.37	74.03
7 MAS	0.00	21.90	36.33	76.00	0.00	22.97	30.90	73.03
8 MAS	0.00	14.40	32.97	70.30	0.00	19.67	29.07	68.37
9 MAS	0.00	6.57	22.53	66.80	0.00	6.70	21.53	66.13
10 MAS	0.00	0.00	20.87	64.37	0.00	0.00	18.87	62.90
11 MAS	0.00	0.00	20.90	64.40	0.00	0.00	18.90	62.90
12 MAS	0.00	0.00	20.20	62.20	0.00	0.00	19.70	61.10
CD	5.84	18.07	15.86	13.65	4.81	12.99	18.44	14.83
F Test	**	**	**	**	**	**	**	**

MAS: Months after storage; \*\* Significant at 1 % level.

Table 2 Viability and germination of Pisifera pollen stored under different temperature regimes

Storage period	Viability (%)				Germination (%)			
	33±2°C	5°C	-5°C	-20°C	33±2°C	5°C	-5°C	-20°C
Fresh	96.70	96.70	96.70	96.70	94.53	94.53	94.53	94.53
1 MAS	72.73	94.13	94.23	95.50	77.07	71.80	81.67	90.33
2 MAS	56.13	60.50	71.50	87.73	62.50	74.83	89.03	92.33
3 MAS	33.03	50.60	80.03	90.90	32.53	50.90	78.87	90.93
4 MAS	21.63	43.47	78.10	83.30	32.47	47.30	65.27	94.87
5 MAS	10.93	47.37	70.87	90.47	5.07	43.63	69.87	90.50
6 MAS	10.47	46.40	66.37	89.20	8.50	42.03	68.47	90.20
7 MAS	9.27	45.87	66.00	88.93	8.77	41.77	68.20	90.07
8 MAS	9.57	40.80	66.77	86.83	9.57	38.07	65.67	86.03
9 MAS	0.00	32.83	61.63	82.57	0.00	31.53	61.07	82.23
10 MAS	0.00	27.70	55.53	76.73	0.00	27.33	53.37	74.10
11 MAS	0.00	27.70	55.50	76.70	0.00	27.30	53.40	74.10
12 MAS	0.00	22.30	52.60	73.50	0.00	20.70	52.50	73.30
CD	10.25	7.84	17.03	8.55	7.58	17.17	22.73	7.79
F Test	**	**	**	**	**	**	*	**

MAS : Months after storage; \*\* Significant at 1 % level; \* significant at 5 % level.

of crapemyrtle pollen when stored at room temperature (23±1) and under refrigerated conditions (4,-20 and -80°C).

In case of storage of pollen at -5°C, though significant reduction was noticed after two months, it was well above the 60% criteria. Pollen viability at two months after storage was on par with that of three months and four months storage. After five months it was significantly different from previous month, whereas it was on par with that of six to nine months

of storage. Pollen viability was around 20% during last three months of storage. At this temperature, germination at two months storage was on par with germination of fresh sample. After four months of storage, the pollen germination was below optimum and it continued to decline, though on par up to 12 months.

After one month of storage at -20°C pollen had viability on par with that of fresh sample. The viability of

pollen significantly declined after two months and was on par with viability up to eight months. After eight months storage, pollen retained more than 70% viability which later declined more than 60% of pollen retained viability at 12 months after storage. The germination was significantly greater in the case of pollen stored at  $-20^{\circ}\text{C}$  than at other temperatures. Although germination of pollen stored at  $-20^{\circ}\text{C}$  gradually declined at every month, more than 60% of pollen germinated at 12 months after storage. Hence, this can be reliable temperature at which pollen of Dura can be stored for up to one year. Towill and Walters (2000) reported extended pollen longevity under temperatures such as  $5^{\circ}\text{C}$ ,  $-20^{\circ}\text{C}$  and  $-80^{\circ}\text{C}$ . According to Mishra and Shivanna (1982) pollen viability differed considerably among species and genotypes at storage temperatures of  $5^{\circ}\text{C}$ ,  $-20^{\circ}\text{C}$  and  $-80^{\circ}\text{C}$ .

#### Longevity of *Pisifera* Pollen

At room temperature, pollens retained their viability up to two months and thereafter declined rapidly. There was significant reduction in viability of *Pisifera* pollen after one month. At two months after storage, viability was just sufficient to consider it as fit for pollination. Afterwards it was below optimum value and declined till eight months after storage. Pollen viability was on par during five to eight months. After that no viable pollen was observed in the sample. Similarly, pollen stored at room temperature retained their germination capacity up to two months and there after declined significantly. Germination of pollen stored at RT was sufficiently high up to two months after storage. Afterwards, it drastically declined and reached around 30 percent during third and fourth months after storage. Further, it declined significantly to record less than 10 percent during five to eight months after storage. The germination was zero during nine

month onwards. Jauh (2002) proposed that some proteins and mRNAs which are necessary for pollen germination tube growth are disintegrated during storage.

At  $5^{\circ}\text{C}$ , at one month after storage the pollen viability declined but was on par with that of fresh. At two months after storage although pollen viability significantly declined than previous month but viability remained sufficient to consider it as fit for pollination. At three months after storage 50% of pollen remained viable which continued to decline and about 20% pollen remained viable after 12 months of storage. Germination percent during one and two months after storage was on par and was significantly higher than that of pollen stored for three months. Pollen germination capacity gradually declined to 20% after 12 months of storage.

In the case of pollen stored at  $-5^{\circ}\text{C}$ , viability at one month was on par with fresh sample. Afterwards pollen viability declined significantly, but 50% of pollen remained viable after 12 months of storage. The germination of pollen stored at  $-5^{\circ}\text{C}$  remained more than 60% up to nine months and declined further to little more than 50% at 12 months after storage.

At  $-20^{\circ}\text{C}$ , around 90% of pollen retained their viability

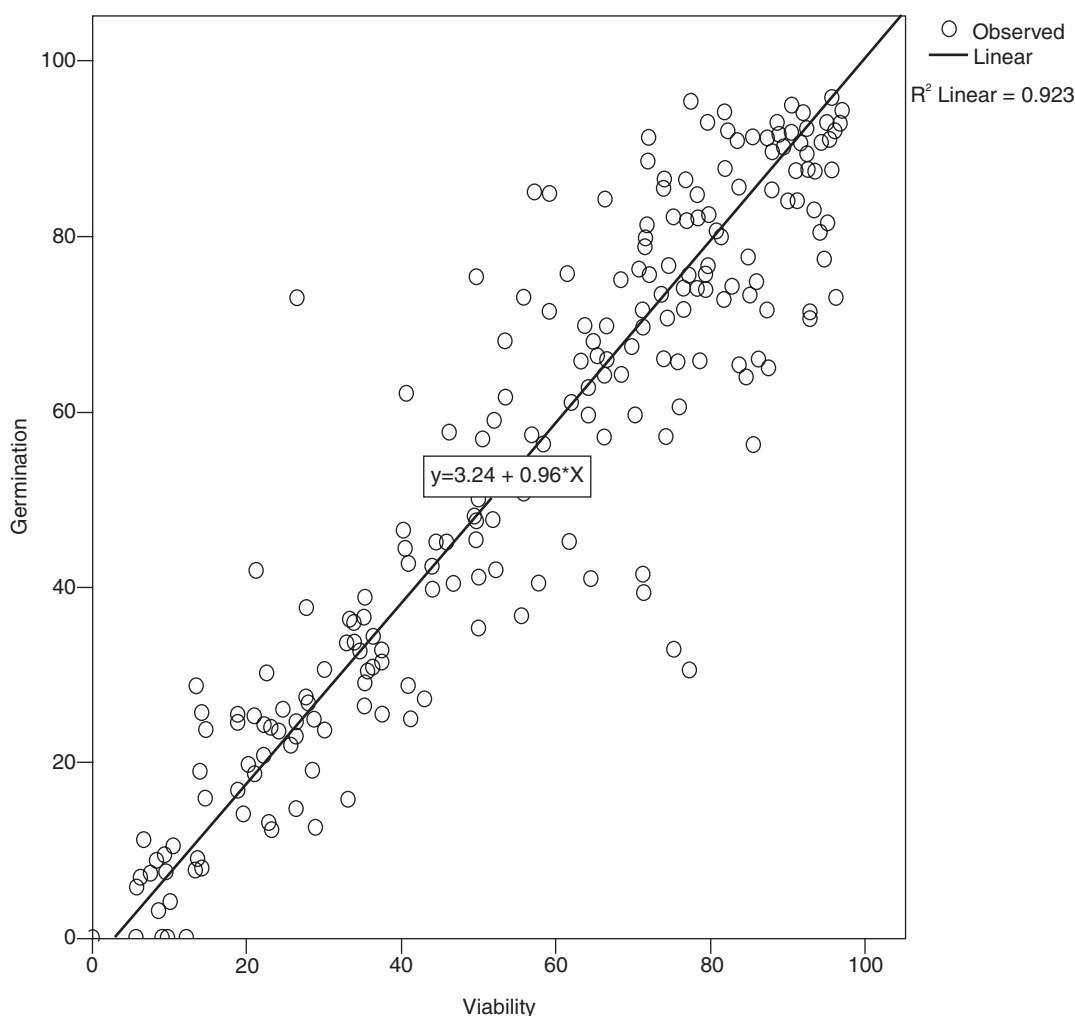


Fig 1 Linear regression of viability and germination

up to six months. Although, pollen viability declined a little every month, more than 70 % of pollen retained their viability after 12 months. This indicated that at -20°C, Pisifera pollen can be stored well beyond 12 months without affecting longevity. The germination of pollen was significantly higher up to nine months after storage. Then it gradually declined but remained above 70 % after 12 months. Akond *et al.* (2012) reported that *Lagerstroemia* pollen was satisfactorily stored up to 75 days or more at -20 °C.

*Correlation of in vitro viability and germination values*

The correlation between *in-vitro* viability and germination values is a measure of accuracy of the estimates and hence, the correlation was worked out (Fig 1). Helpson-Harrison *et al.* (1984) reported that a close correlation between FCR test and germinability can only be expected when the pollen is mature and the medium is optimum. Staining test alone can be misleading due to over estimation of fake positives. Over estimation of similar types was reported in Mango (Choudhury *et al.* 2010, Shivanna and Helpson-Harrison 1981). The correlation obtained between viability and germination was 0.96 which indicates that the methodologies adopted were accurate and the parameters were estimated precisely.

*Longevity of Dura vs Pisifera*

At room temperature the longevity of Dura and Pisifera pollen significantly differed. Pisifera pollen could retain greater viability as well as germination than Dura at all the temperatures of storage. Viability of Dura pollen declined drastically after two months of storage whereas pollen of Pisifera remained viable for much longer period. Viability of Dura pollen declined below 10% after two months, whereas Pisifera reached same level after five months. At -20 °C also, similar variation was observed for viability of Dura and Pisifera pollen. When pisifera retained close to 90% viability after two months, Dura pollen retained only about 75%. Similar trend was continued till 12<sup>th</sup> month where Pisifera retained close to 75 percent viability and Dura just above 60 percent. This implies that Dura pollen can be stored up to one year and Pisifera pollen beyond one year at this temperature.

A comparative study on viability and germinability

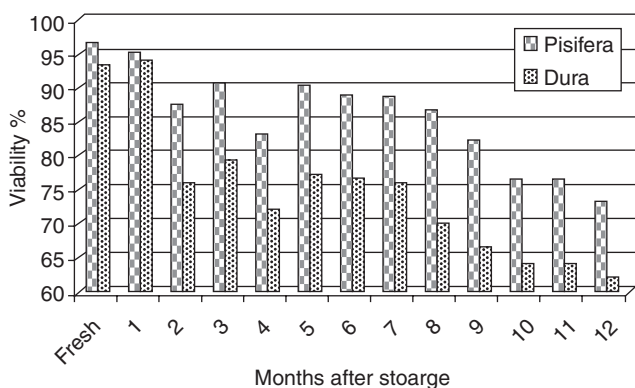


Fig 2 Viability of Dura vs Pisifera pollen stored at -20°C

of pollen in three mango cultivars indicated that these parameters are genotype dependant (Albuquerque *et al.* 2001). Similar findings were reported by Abd El-Hadi (2006). Bomben *et al.* (1999) observed a rapid and linear decrease in germination of pollen stored at 4°C than at -18°C along with divergent response of species.

*In vivo fruit set studies*

To confirm the results of *in vitro* assessment of viability and germination, fruit set studies were carried out *in vivo* using pollen stored for six months (Table 3 and 4). In case of Pisifera pollen, bunch analysis data revealed that mean maximum bunch weight was obtained with pollen stored at -20°C and the lowest in the case of pollen stored at room temperature. Weight of fruits (after removing stalk and

Table 3 *In vivo* fruit set and related bunch parameters of Pisifera pollen stored for 6 months

Bunch parameters of Dura × Pisifera cross	Storage temperatures			
	33±2°C	5°C	-5°C	-20°C
Bunch weight (kg)	13.32	17.3	15.65	19.17
No. of sterile fruits	520	379	193	97
Total no. of fruits	1683	2075	1037	1772
% of sterile fruits	30.90	18.27	18.61	5.47
Total fruit wt (kg)	9.09	11.47	11.01	13.9

Table 4 *In vivo* fruit set and related bunch parameters of Dura pollen stored for 6 months

Bunch parameters – Dura × Dura	Details of storage temperatures			
	33±2°C	5°C	-5°C	-20°C
Bunch weight (kg)	10.25	11.57	17.05	22.23
No. of sterile fruits	560	344	287	247
Total no. of fruits	1734	1037	1293	1821
% of sterile fruits	32.29	33.17	22.19	13.56
Total fruit wt (kg)	6.31	3.37	12.35	16.33

spikelet rachis) was the maximum for pollen stored at -20°C followed by pollen stored at -5 and 5°C. The pollen stored at room temperature resulted in lesser fruit weight. Further, percent sterile fruits was the maximum in the case of pollen stored at room temperature and the minimum in the case of pollen stored at -20°C, indicating the effectiveness of the latter treatment for fruit set by way of retaining longevity of the pollen. Akond *et al.* (2012) reported satisfactory seed set by using *Lagerstroemia* pollen stored at -20°C.

Similarly, *in-vivo* fruit set study was carried out using stored Dura pollen. The bunch parameters indicated that the maximum bunch weight was recorded by pollen stored at -20°C. This was followed by pollen stored at 5°C and -5°C. The lowest bunch weight was obtained with pollen stored at room temperature. Similar was the trend for total fruit weight where the maximum being in pollen stored at -20°C and the least in the case of 5°C followed by room

temperature. The percent sterile fruit was the maximum in the case of pollen stored at room temperature which was on par with 5°C. The lowest sterile fruit percent was in the case of pollen stored at -20°C and -5°C being the next best storage temperature.

Dura pollen retained viability for one month whereas Pisifera for two months at room temperature storage. At 5°C, Dura pollen retained viability up to two months, whereas Pisifera for three months. At -5°C, Dura pollen remained viable up to four months. But Pisifera pollen retained viability up to nine months. When stored at -20°C, both Dura and Pisifera pollen remained viable for 12 months, with Pisifera retaining higher percentage than Dura. The overall viability and germination decreased with increased storage period as well as with storage temperature. Temperatures above 0°C was not suitable for storage of pollen from both fruit form types. The results of the study pointed that -20°C was the best storage temperature for both the fruit forms for up to one year. The viability retention of Pisifera beyond one year could be further investigated to optimize the duration of storage. To the best of our knowledge, this is the first report on differential pollen longevity in the case of oil palm Dura and Pisifera fruit types.

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