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Effect of low temperature storage on oil palm (*Elaeis guineensis Jacq*) seed viability

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ABSTRACT: *Oil palm (*Elaeis guineensis *Jacq.) is the highest edible oil yielding (5- 8 tonnes per ha) perennial crop. Commercially it is propagated through seed. In the processing of hybrid seed leading to development of commercial tenera planting material owing to seasonal maxima in the production of fruit bunches, glut in the production of seed sprouts is a common occurrence in all the seed labs. This necessitates storage of oil palm seed before processing them for development of seed sprouts. Further cryo-preservation (storage at very low temperature) is the established method for long term conservation of germplasm in many crops. In this backdrop, an attempt is made to find out the effect of low temperature (-4, -20 -40 °C, 23 °C and ambient temperature (uncontrolled room temperature) storage for different durations (15, 30, 45, 60, 75 and 90 days) on the viability of oil palm seed. Seed viability measured through tetrazolium staining test was found to lost/disappear when stored at sub-freezing temperatures (-4, -20 and -40 °C) irrespective of the storage duration. Storage at 23 °C or ambient temperature did not affect the viability even after 90 days. It appears that low temperature storage (at sub-freezing temperatures) is not suitable for oil palm seed. The biochemical reactions like fall in the levels of sugars & proteins and the acceleration of lipid peroxidation, however, appears to commence after 60 days of storage. Fresh oil palm seed can be stored up to 90 days at 23 °C without any loss in viability.*

Key Words: *Oil palm, seed viability, lipid peroxidation, seed storage, sub-freezing temperature, orthodox, recalcitrant*

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is the highest edible oil yielding (5- 8 tonnes per ha) perennial crop. Oil palm is commercially propagated through seed (hybrid seed from the cross of Dura, D x Pisifera, P) (Corley and Tinker, 2003). Seeds which have a dormancy or rest period for about 2 - 3 months are germinated under controlled condition and the sprouts are transported to desired locations where the seedlings (up to 12 – 14 months of age) will be raised in a nursery. Vegetative means of propagation including tissue culture is not yet standardized in India.

In India oil palm hybrid seed production has been started in the exclusive 'Oil Palm Seed Gardens' established in different parts of the country from time to time. At present there are six seed gardens, DOPR (ICAR), RC, Palode (near Trivandrum, Kerala), DOPR (ICAR), Pedavegi (near Vijayawada, A. P.), Dept of Hort, Govt of A. P., Rajahmundry, Dept of Hort, Govt of Karnataka, Taraka, Oil Palm India Limited, Thodupuzha (a joint venture of Governments of India and Government of Kerala State) and M/s Navabharat Agro Products Limited, Lakshmipuram, West Godavari Dist., A.P. producing tenera sprouts for commercial planting (Naveen Kumar *et al*., 2013). Delay in lifting of oil palm sprouts from these seed gardens leads to overgrowth of the sprouts and increase in abnormalities due to which the whole lot of sprouts have to be discarded.

In the processing of hybrid seed leading to development of commercial tenera planting material, glut in the production of seed sprouts is a common occurrence in all the seed labs owing to seasonal maxima in the production of fruit bunches. This necessitates storage of oil palm seed before processing them for development of seed sprouts. Conserving the oil palm genetic resources in *ex situ* living collections requires high cost of maintenance, large land area and the palms are exposed to diseases and extreme weather conditions. As regards *in vitro* conservation possibilities, oil palm was classified

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recently as being neither recalcitrant nor orthodox, but showing intermediate seed storage behaviour (Ellis *et al*., 1991). It was reported that intermediate seeds can be desiccated to around 10-12% moisture content and can tolerate freezing temperatures.

Seeds of a large number of tropical, subtropical and temperate species have been termed recalcitrant (Roberts 1973) since they are sensitive to desiccation and can thus be conserved for short periods only (weeks-months) even in the optimal moisture conditions. Careful adjustment of the storage environment (humidity, temperature) led to improvements in the conservation duration for several of these species such as oil palm and coffee (Ellis *et al*. 1990, 1991) which are now considered intermediate in their seed storage behaviour. Nevertheless, long-term storage of these seeds still remains impossible.

Cryopreservation method offers minimum space and low maintenance and has become very important tool for long term storage of germplasm materials. It appears to be the most feasible method for storing recalcitrant seeds and species that are vegetatively propagated. Several workers reported that the excised embryos are relatively higher tolerant to dessication and cryoexposure than whole seeds in recalcitrant and intermediate species (eg. oil palm) (Normah et al., 1994 and Makeen et al., 2005). The practical problems in oil palm include excision of embryo from the seed and non-availability of repeatable protocol for embryo culture *in vitro*. Hence, an attempt was made to find out the effect of sub-freezing temperatures low temperature (-4, -20 and -40 °C along with ambient (uncontrolled) and a constant room temperature of 23 °C) storage for different durations (15, 30, 45, 60, 75 and 90 days) on the viability of oil palm seed.

MATERIALS AND METHODS

Fresh oil palm dura seeds, immediately after processing (depericarping and shade drying) were stored at five different temperatures, -4, -20, -40°, 23 °C and ambient (uncontrolled) for different durations (15, 30, 45, 60, 75 and 90 days). At the end of each storage duration, moisture and oil content, total soluble sugars, proteins, lipid peroxidation and viability were worked in all the treatments. Moisture content was determined based on the difference in initial and final weights. Whereas oil content was worked out using soxhlet's apparatus as suggested by Mandal and Gayathri (2005). Triphenyltetrazolium chloride test as suggested by Lakon (1949) was conducted to test the seed viability.

Total soluble sugars were estimated as per the procedure suggested by Hedge and Hofreiter, (1962). Total soluble protein content was assayed by Lowry *et al.* (1951) based on the observation that the absorbance maximum for an acidic solution of Coomassie Brilliant Blue G-250 shifts from 465 nm to 595 nm when binding to protein occurs. Both hydrophobic and ionic interactions stabilize the anionic form of the dye, causing a visible color change.

The level of lipid peroxidation was measured in terms of Thio barbutaric acid a product of lipid peroxidation following the method of Health and Packer (1968). Centrifugation at 10,000 g for 10 minutes to remove suspended turbidity the absorbance of the supernatant was recorded at 532 nm. The value for non specific absorption at 600 nm was subtracted. The thio barbutaric acid content was calculated using its absorption coefficient of 155 m mol^{-1} cm^{-1}.

RESULTS AND DISCUSSION

The effect of low temperature (-4, -20 and -40° and 23 °C and ambient (uncontrolled)) storage for different durations (15, 30, 45, 60, 75 and 90 days) on oil palm seed viability was interpreted based on the following results.

Moisture and Oil Content

Rees (1963) concluded that the optimal moisture content for long term storage of oil palm seed is between 14.5 and 22%. In the present investigation, storage at ambient or 23 °C seed moisture content resulted in decrease in moisture content gradually with increase in storage duration (Table 1). Storage at sub-freezing temperatures (-4, -20 & -40) did not show consistent trend but it appears that these treatments could maintain higher moisture content than the ambient or 23 °C. It appears that seed moisture content over a certain limit has no influence on the viability of seeds. This is supported by the findings of Ellis *et al*., (1991) who demonstrated the orthodox behaviour of oil palm seed by successfully storing the tenera seed dried to 7-10% moisture content. On the other hand, rehydration to rise the MC to >20% could have improved the seed viability as suggested by Mok (1982).

Oil content showed reverse trend in the seeds stored at ambient temperature (Table 2). Average oil content was highest in this treatment (34.9) but there appears not much difference in the oil content recorded in other treatments which gives us scope to say that the lack of oil (fatty acid) reserves is not the

cause for loss in viability.

Seed Viability

Based on the intensity of staining, an OD (absorbance) value of 0.4 at 484 nm was considered as the bench mark to group the seeds viable (OD > 0.4) and non-viable (OD< 0.4). All the seeds stored at 23 °C or ambient (room temp) were found to retain the viability even after 90 days storage (Table 3). Conversely, storage at sub-freezing temperature resulted in loss of viability irrespective of the duration of storage. Wood *et al.* (2005) while estimating the seed viability using the topographical tetrazolium (TZ) stain with germination data for 171 species from 27 families stated that TZ consistently underestimated viability compared with germination testing for species with oily seeds (typically ca. 20-30%). Viability and germination data were more comparable in the species which typically have low oil contents (ca. 5-8%). They suggested that there's need for TZ protocol improvements, especially for high oil content seeds.

In the present study, seed storage at 23 °C was found to be better which is in conformation with the findings of Rees (1965) who reported that storage at 22^0C gave good results. Corley and Tinker (2003), however, summarized that storage at 20-22 °C with a moisture content of 18-19% (not below 16%) is beneficial for oil palm seed. Thus, although, oil palm seed is grouped as intermediate, recalcitrant behavior is more prominent. Accordingly, in the present study also, rehydration (to >16%) could have yielded beneficial results at sub-freezing temperatures.

Soluble Sugars and Proteins

Soluble carbohydrates have been proposed to play an important role in conferring desiccation tolerance and storability in seeds. In model systems, sucrose has been shown to protect the structure and function of desiccated phospholipids (Hoekstra, Crowe and Crowe, 1989). In the present investigation, levels of total soluble sugars in all the treatments increased gradually with increase in storage duration up to 60 days but then increased sharply at 75 days and thereafter declined at 90 days (Table 4). The magnitude of sugar levels was, however, highest in the ambient temperature (room temp) at 75 days and at 23 °C (at 90 days). Increases in levels of sucrose and particularly the raffinose family oligosaccharides have been correlated with the onset of desiccation tolerance during orthodox seed development (Blackman, Obendorf and Leopold, 1992). Similarly, the loss of these carbohydrates at the start of germination has been found to coincide with the re-acquisition of desiccation sensitivity (Leprince *et al.*, 1992). Steadman *et al.*, (1996) reported that the accumulation of specific soluble carbohydrates has been implicated in the acquisition of desiccation tolerance and improved longevity in orthodox seeds, leading to the hypothesis that carbohydrate composition might be used as a diagnostic marker for seed storage category.

Total soluble protein content also showed similar trend of gradual increase up 60 days of storage followed by sharp decline at 75 and 90 days. The magnitude of protein levels was, however, highest in the ambient temperature (room temp) followed by at 23 °C for all storage durations (Table 5). The difference (initial and final) in the level of sugars and proteins showed a significant fall with decrease in storage temperature (Fig 1 A & B).

Lipid Peroxidation

Decline in the content of membrane phospholipids is a key event in senescence. In the present study, lipid peroxidation measured in terms of thiobarbutric acid active substance (TBARS) increased at the end of the storage (90 days) showing decline in phospholipids

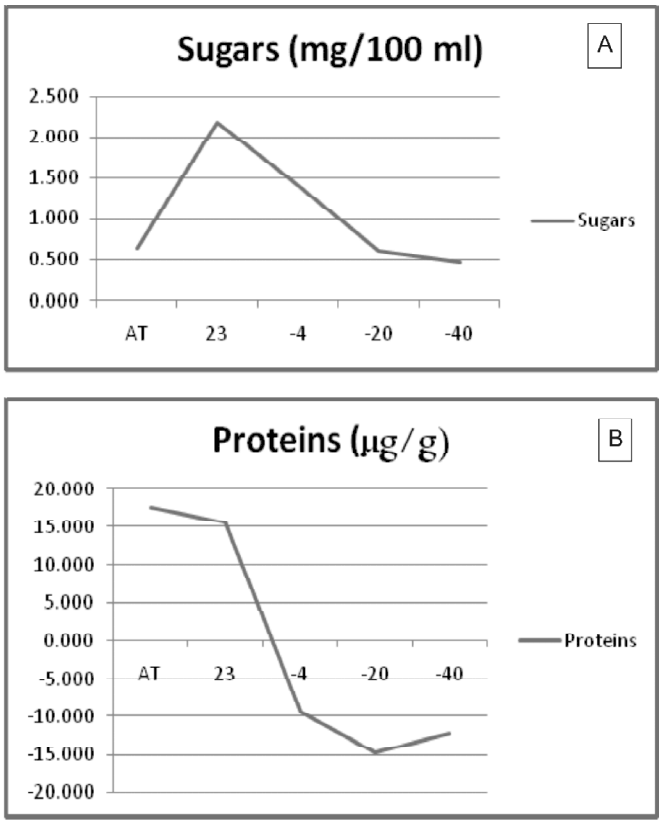

Figure 1: Effect of low temperature storage on total soluble sugars (A) and proteins (B) in oil palm (dura) seed.

Tabel 1
Effect of low temperature storage on loss of moisture in oil palm seed

Storage duration (days)	*15*	*30*	*45*	*60*	*75*	*90*
Storage temperature (°C)						
Ambient (Room Temp)	12.62	12.10c	10.78c	10.15e	9.84c	11.58
23	12.89	13.17b	11.80b	11.27d	11.84b	11.38
-4	13.13	14.20a	13.86a	12.65c	14.38b	19.01
-20	13.41	13.38ab	14.32a	13.34b	12.46a	11.90
-40	12.42	14.00ab	13.88a	14.07a	14.42a	13.03
CD (P=0.05)	NS	0.87	0.76	0.31	0.87	NS

Tabel 2
Effect of low temperature storage on oil content of oil palm (tenera) seed.

Storage duration (days)	*15*	*30*	*45*	*60*	*75*	*90*	*Average*
Storage temperature (^{0}C)							
Ambient (Room Temp)	24.15d	47.65a	35.74a	31.98	34.04a	36.07a	34.9
23	31.97b	29.28d	24.00b	32.03	30.50b	26.75bc	29.09
-4	31.96b	34.64c	27.02b	28.02	19.50c	31.26ab	28.73
-20	28.56c	35.67bc	25.19b	33.58	29.84b	23.89c	29.46
-40	37.32a	37.07b	19.70c	24.92	18.66c	30.73ab	28.07
CD (P=0.05)	1.25	1.73	3.49	NS	2.13	6.15	

Tabel 3
Effect of low temperature storage on viability of oil palm (tenera) seed

Storage duration (days)	*15*	*30*	*45*	*60*	*75*	*90*
Storage temperature (^{0}C)						
Ambient (Room Temp)	V	V	V	V	V	V
23	V	V	V	V	V	V
-4	NV	NV	NV	NV	NV	NV
-20	NV	NV	NV	NV	NV	NV
-40	NV	NV	NV	NV	NV	NV

V- viable and NV – non-viable

Tabel 4
Effect of low temperature storage on total soluble sugars (mg/100 ml) in oil palm (dura) seed

Storage duration (days)	*15*	*30*	*45*	*60*	*75*	*90*
Storage temperature (^{0}C)						
Ambient (RT)	1.43b	1.55	1.85ab	1.89c	5.34a	2.08c
23	1.42b	1.60	1.78bc	1.85cd	4.50b	3.60a
-4	1.43b	1.56	1.85ab	1.81d	3.30c	2.84b
-20	1.38c	1.61	1.67c	2.06b	3.17c	2.01c
-40	1.51a	1.47	1.93a	2.41a	3.49c	1.98c
CD (P=0.05)	0.039	NS	0.109	0.081	0.356	0.712

Tabel 5
Effect of low temperature storage on total soluble proteins (µg/g) in oil palm (dura) seed.

Storage duration (days) Storage temperature (^{0}C)	*15*	*30*	*45*	*60*	*75*	*90*
Ambient (RT)	39.19bc	66.64	134.25	157.38a	82.33a	56.73a
23	39.68b	59.02	99.96	150.27a	86.47a	55.20a
-4	39.19bc	59.27	55.20	125.87b	62.83b	29.79b
-20	35.12c	66.90	66.39	145.69a	66.39b	49.87a
-40	44.78a	51.14	73.50	110.11b	62.07b	32.58b
CD (P=0.05)	4.21	NS	10.83	19.02	4.85	7.23

Table 6
Effect of low temperature storage on lipid peroxidation (n mol/g) in oil palm (dura) seed

Storage duration (days)	*15*	*30*	*45*	*60*	*75*	*90*
Storage temperature (^{0}C)						
Ambient (RT)	0.078	0.022	0.02	0.018bc	0.021b	0.024cd
23	0.039	0.025	0.077	0.016c	0.021ab	0.022d
-4	0.027	0.033	0.02	0.016c	0.023a	0.032b
-20	0.028	0.02	0.023	0.023a	0.013d	0.062a
-40	0.018	0.069	0.02	0.020b	0.019c	0.026c
CD (P=0.05)	NS	NS	NS	0.003	0.002	0.003

(Table 6). The levels, however, were minimum at 60 days storage duration indicating that the onset of senescence is not yet commenced. The effect of different treatments on the extent of lipid peroxidation was significant after 60 days of storage. Lipid peroxidation and lipid metabolites were found to enhance senescence, through regulation of ethylene production or action (Halevy, 1986). At sub-freezing temperatures the levels of TBARS were highest, this may indicate that the process of senescence is accelerated due to the low temperature stress.

In summary, the findings of low temperature effect on moisture and oil content supports the fact that the oil palm seed is intermediate in storage behavior. The results from viability testing coupled with sugars and proteins content indicate that the sub-freezing temperatures are not suitable for long term storage. It appears that the process of senescence gets accelerated due to the low temperature stress which may lead to loss of viability.

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