ISSN: 2287-688X

Annals of
Plant Sciences

Volume 12, Issue 03 (2023) pp. 5773-5782

Research Article

Biochemical Changes during the Seed Storage of *Mesua ferrea* L., A Medicinal Tropical Tree Species

Mithun V[1], Pradeep N.S[1] and P N Krishnan[2]

[1]*KSCSTE-Malabar Botanical Garden and Institute for Plant Sciences, GA College Post, Kozhikode, Kerala, India, 673014*

[2]*Centre for Innovation in Science and Social Action (CISSA), T.C. 15/510, USRA -55, Udarasiromani Road, Thiruvananthapuram - 695010, Kerala, India*

Abstract

Seeds of *Mesua ferrea* L. shows recalcitrant seed storage behaviour and seeds are viable only for 8-15 days after harvest in normal storage condition. *M. ferrea* seeds during storage in open laboratory condition at room temperature lost moisture content (MC) and viability within 15 days on the other hand the seeds stored at 10℃ in closed polycarbonate bottles retained MC and the viability was extended to 180 days. Present paper describes biochemical changes *viz*. total soluble sugar (TSS), starch, total protein, amino acid, lipid and phenol contents during the two storage conditions at different periods together with peroxidase (PO) and superoxide dismutase (SOD) enzyme activities. Increase in the level of TSS, amino acids, lipids, phenols and enzymes PO and SOD recorded during *M. ferrea* seed storage and their role in desiccation tolerance was evaluated.

Keywords: *M. ferrea, viability, biochemical changes, desiccation, germination, moisture content.*

Introduction

Seeds that can be safely dried to moisture contents between 6% and 10% and stored successfully at low temperatures have been described as 'orthodox' in storage behaviour, while seeds that cannot be dried to these levels without losing viability have been described as 'recalcitrant' (Roberts, 1973). Among economically important tree species, seed handling techniques have clearly identified which are orthodox and which are recalcitrant. A characteristic of recalcitrant seeds is the variability they show among species and within a species. They vary in the water content at the time of shedding, the extent of dehydration they tolerate, their response to drying rate, storage lifespan in the hydrated state, and their response to low temperatures (for some examples see Farrant, *et al*., 1989; Hong and Ellis, 1990; Pritchard, 1991; Berjak, *et al*., 1993; Tompsett and Pritchard, 1993) means that it is not simply a case of classifying a seed as orthodox or recalcitrant, but within the recalcitrant group there is a wide spectrum of behaviours, from minimally recalcitrant seeds with a relatively long lifespan and quite tolerant of desiccation, to maximally recalcitrant, with short lifespans and very sensitive to desiccation (Farrant, *et al*., 1988). The long-term storage of recalcitrant seeds remains a challenge in the conservation of rare endangered and threatened species.

Mesua ferrea L. is a medicinal tree species distributed in Indo-Malaysian regions. The species is an evergreen one growing up to 20–30 meters high but it is slow-growing. It is commonly known as iron wood tree. The phenolic compounds present in seeds *viz*. Mesuol ($C_{23}H_{22}O_5$) and Mesuone ($C_{29}H_{42}O_4$) have anti-bacterial properties (Anonymous, 1952) other than glycosides, flavonoids, xanthones, triglycerides, and resins. The plant is used as antimicrobial, antibacterial, and anti-protozoal (Mazumder, *et al*., 2004; Chanda, *et al*., 2013). The flowering process,

***Corresponding Author:**
P N Krishnan;
DOI: http://dx.doi.org/10.21746/aps.2023.12.3.4

seed formation and maturation are critical phases in the life cycle, of which are highly specific to the environmental factors, disturbances and final viability and germination of the seeds they produce. Studies in *M. ferrea* related to fruit size variation, germination, seedling fitness and biomass accumulation during early seedling growth was reported (Khan, *et al*., 2002; Arunachalam, *et al*., 2003). Phenology and seed development in *M. ferrea* were studied by Mithun, *et al*., 2020a. Tree commences to produce fertile seeds at 15 to 20 years of age. Good seed years take place at irregular intervals. In this background conventional as well as non-conventional technologies are need to be developed for conservation of this economically and medicinally important species. In this paper we report the conventional seed storage studies conducted in *M. ferrea* in laboratory condition and closed polycarbonate bottles kept at 10° C.

Recalcitrant seeds are metabolically active at the time of shedding and are ready for quick germination. This is evidenced by respiration measurements and ultra-structural studies of mature seeds (Farrant, *et al*., 1988). On dehydration a wide spectrum of abnormalities occur in the seeds. Among this, the generation of highly reactive free radicals of oxygen creates major problems. These radicals cause the peroxidation of membrane lipids; and there by leading to leakage of solutes from the seed tissues. For combating the lethal effects of free radicals like O_2-, OH-etc the seeds are equipped with enzymatic and non-enzymatic protection mechanisms. Oxygen-dependent reactions occur in dry seeds and alter cellular components leading to damaging reactions (Reuzeau and Cavalie, 1995). Most of the free radicals released during such reactions are de-toxified by scavenging enzymes like peroxidase, poly phenol oxidase, super oxide dismutase, catalase etc. The study on the activity of free radical scavenging enzymes may help in ascertaining the level of desiccation tolerance and will also help to identify the safe moisture content of the seeds. It is at this critical moisture content that the seeds can be stored for a longer period than the fresh seeds. As *M. ferrea* become economically important in timber, medicine and ornamental, no effort has been taken to seed storage on the line of conservation. In this paper we describe the seed germination and storage with respect to two different storage conditions and related biochemical and free radical scavenging enzymes peroxidase (PO) and superoxide dismutase (SOD) changes during desiccation and storage.

Materials and Methods

Collection of Seeds

Mature fruits of *M. ferrea* are collected from the selected trees grown in Calicut University Campus, Malappuram by gentle shaking of fruit bearing branches. The fruits were collected in baskets and not let fall on the ground to avoid damage. As the seed viability depends on the harvest time the seed collection was made only after the complete maturity of the seeds. In the laboratory fruits were washed in running tap water for one hour to remove the debris, other fruit exudates and blotted dried using blotting paper. Then seeds dissected out from the fruits were surface sterilized with 0.1% mercuric chloride for 10 minutes and spread on an absorbent paper for drying. Blotted dried seeds were stored in two different conditions as follows: Storage conditions used for the present study are:

1) Open laboratory conditions (28±2℃ & 65%RH) in plastic trays (OLC)
2) Closed polycarbonate bottle at 10℃ (CPBo10℃)

Biochemical Changes during Storage

Stored seed samples were taken at an interval of 5 days and tested for % MC, viability as germination percentage, enzyme activity of PO, SOD and biochemical changes of TSS, protein, starch, amino acids, lipids, phenols during storage.

Moisture content of the seeds at each interval was determined according to ISTA rules (2015), High Constant Air Owen method, *i.e*., by drying at 130±1℃ for 1 hour.

Metabolites including total sugars, phenolics, amino acids, protein, lipids and starch at each interval were analysed from the samples collected at different periods were dried in an owen at 80°C for 48 hrs. These samples was ground in known volume of 80% ethanol (v/v) in distilled water and centrifuged at 4000 rpm for 10 minutes. The residue was washed thrice and part of the combined supernatant used for the estimation of total sugar, phenol and amino acids. The rest of the supernatant was kept in a China dish and evaporated in a hot air oven at 60°C and the residue was dissolved in distilled water, centrifuged and served as the source for soluble sugar. The left-over residue was ground in 30% perchloric acid, centrifuged, re-extracted twice and the combined supernatant is used for starch estimation. Total soluble sugar was estimated using phenol sulphuric acid method (Montgomery, 1957), total phenols by Swain and Hillis, 1959; protein content by Lowry, *et al*., 1951, starch by Mc Cready, *et al*., 1950; amino acid by Sadasivam and Manickam, 1996 and lipids by the method of Bligh and Dyer, 1959.

Extraction and Assay of Enzymes

One-gram fresh seed tissue collected during different storage periods was homogenised in a pre-chilled mortar and pestle with a pinch of purified sand and 0.1 M cold sodium phosphate buffer (pH 7.0). The homogenate was centrifuged at 5000 rpm for 20 minutes using refrigerated centrifuge and the supernatant was collected. Proteins from the supernatant was precipitated in cold acetone twice and re-suspended in the extraction buffer after the evaporation of the acetone. The extract was centrifuged and the supernatant served as the enzyme source for both peroxidase and polyphenol oxidase assay. All the extraction procedures were carried out at 4^0C.

Peroxidase enzyme activity was measured according to the method of Chance and Maehly, 1965 by recording the change in absorbance at 470 nm due to oxidation of guaiacol in presence of hydrogen peroxide. The assay mixture (3 ml) consisted of 1 ml 0.01 M sodium phosphate buffer (pH 7.0), 0.5 ml 0.02 M guaiacol and 1 ml enzyme extract. The reaction were triggered by the addition of 0.5 ml 13 mM H_2O_2 to the mixture. The change in absorbance was recorded in spectrophotometer (Systronics model 106) at 470nm. The enzyme activity was measured as change in absorbance per minutes.

Superoxide dismutase (SOD) activity was determined by measuring the inhibition in photoreduction of nitroblue tetrazolium (NBT) by SOD enzyme ((Zheng, *et al*., 2015). The reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 0.1 mM EDTA, 50 mM sodium carbonate, 12 mM L-methionine, 50 µM NBT, 10 µM riboflavin and 100 µL of crude extract in a final volume of 3.0 mL. A control reaction was performed without crude extract. The SOD reaction was carried out by exposing the reaction mixture to white light for 15 min at room temperature. After 15 min incubation, absorbance was recorded at 560 nm using a spectrophotometer. One unit (U) of SOD activity was defined as the amount of enzyme causing 50% inhibition of photochemical reduction of NBT.

Statistical Analysis

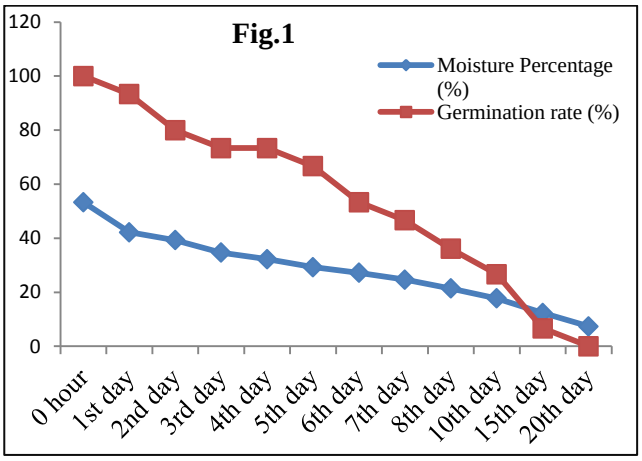
The data of % moisture content and % germination as a function of storage days were analysed statistically following linear regression analysis using the formula Y= a + bx. Correlation coefficient "r" have been calculated and the significance as per Sokal and Rohlf, 1981.

Results

Seed Moisture Content and Germination during Storage

In general, germination percentage and moisture content of the seeds decreased with the increasing days of storage in both the cases. During the harvest time the moisture content of the seeds was at the range of 53-55%, indicate the higher moisture content and 100% germination. This indicates the recalcitrant behaviour of the seeds which lose viability within the short time and a reduction of 10% of MC from the initial MC. Seeds stored in open laboratory condition (OPL) recorded a sharp decrease in % MC from

53.35% to 7.39% and germination percentage from 100% to 0% within 20 days respectively (Fig. 1). While the seeds stored at closed polycarbonate bottle and stored at 10°C (CPBo10°C) recorded a slower rate of decrease in MC from 53.24% at 0 days to 34.68% on 180 days of storage (Fig. 2). Similarly, the viability of the seeds was also retained till 150 days of storage (21.48%) (Fig. 2) which was about 10 times greater than that of OPL stored seeds. The pattern of moisture lost and germination against the storage period were analysed statistically regression analysis and the patterns were significant at 0.01% P level for both the cases (Table 1).



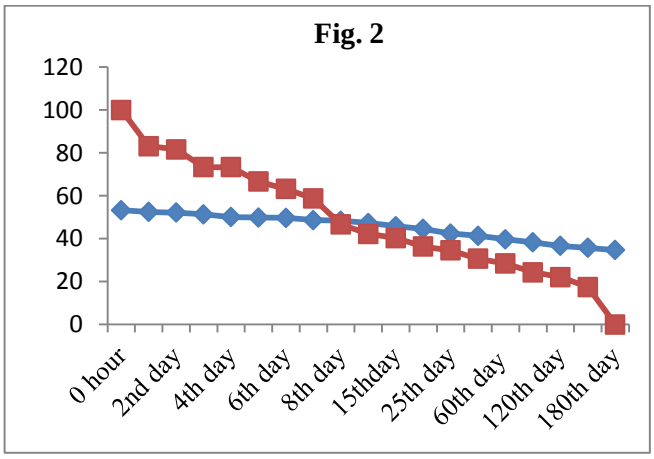


Fig.1: Changes in % MC and Germination of *Mesua ferrea* seeds stored in Open laboratory condition
Fig. 2: Changes in % MC and Germination of *Mesua ferrea* stored in Polycarbonate bottle at 10℃

Table 1: Linear regression analysis: Seed germination and % Moisture content as a function of storage days

Variable	Formula	Degree of freedom	r
Fig.1 Storage vs Germination	y = -5.3216x + 90.764	12-2=10	0.9660[a]
Fig. 1 Storage vs % MC	y = -2.0664x + 42.433	12-2=10	0.9353[a]
Fig.2 Storage vs Germination	y = -0.4766x + 85.583	24-2=22	0.9315[a]
Fig. 2 Storage vs % MC	y = -0.0954x + 48.888	24-2=22	0.8883[a]
[a]Significance at 0.01% P level			

Biochemical Changes

Total soluble sugar (TSS) content increased linearly on increasing of storage days in both the storage conditions (Table 2 and 3). In OPL the TSS was increased from 68.94 ± 7.4 mg^{-1} d. wt. to 107.33 ± 7.5 mg^{-1} d. wt. 20 days where complete loss of viability was recorded. Whereas TSS in seeds stored CPBo10°C recorded maximum on 180 days 120.56 ± 8.72 mg^{-1} d. wt. On the other hand, starch content showed a decreasing trend with increasing desiccation in both the conditions (Table 2 and 3). A decreasing trend in total protein content with an increase in storage period was recorded with both storage conditions whereas a gradual increase in amino acid content was recovered with increasing storage period (Table 2 and 3). Lipid content of fresh seeds (465.29 ± 5.79 mg^{-1} d. wt.) was decreased on desiccation or storage. Total phenol content recorded an increase with increasing days of storage from an initial 38.64 ± 2.04 mg^{-1} d. wt. to 54.29 ± 4.4 and 60.11 ± 3.06 mg^{-1} d. wt. in OPL and CPBo10°C stored seeds respectively (Table 2 and 3).

Table 2: Changes in metabolites during storage of *Mesua ferrea* seeds in open laboratory condition

Storage days	Sugars (mg-1d.wt.)	Starch (mg-1d.wt.)	Total protein (mg-1d.wt.)	Amino acids (mg-1d.wt.)	Total lipids (mg-1d.wt.)	Phenols (mg-1d.wt.)
0	68.94±7.4	140.48±10.1	90.21±7.3	3.78 ± 0.09	465.29 ± 5.79	34.23±2.9
1	67.12±6.9	133.69±9.5	88.34±8.6	3.83± 0.08	460.37 ± 5.18	36.04±3.9
2	69.84±7.1	124.37±10.6	80.39±7.9	3.87 ± 0.06	454.32 ± 5.42	39.48±3.5
3	67.97±5.3	119.34±8.9	77.97±6.7	3.90 ± 0.14	448.93 ± 4.78	37.66±3.1
4	70.23±4.9	100.12±9.8	76.94±7.2	3.92 ± 0.08	440.21 ± 3.91	40.28±2.9
5	72.36±6.9	95.06±8.4	69.10±6.8	3.95 ± 0.16	433.85 ± 5.18	42.13±3.9
6	82.69±7.9	86.97±8.6	64.29±5.9	3.98 ± 0.12	429.18 ± 5.32	44.54±3.6
7	87.42±7.9	82.01±7.9	59.33±4.8	4.09 ± 0.16	420.77 ± 4.23	47.22±4.1
8	91.28±8.1	70.88±6.4	54.20±6.1	4.19 ± 0.10	404.28 ± 3.89	49.59±4.6
10	96.47±8,9	63.29±5.9	50.14±4.8	4.23 ± 0.22	389.58 ± 4.06	50.08±4.2
15	105.39±9.9	57.44±4.9	42.67±3.9	4.31 ± 0.18	378.15 ± 3.52	51.37±4.7
20	107.33±7.5	48.94±3.7	37.41±3.1	4.46 ± 0.16	366.73 ± 3.89	54.29±4.4

Table 3: Change in metabolites during storage of *Mesua ferrea* seeds in closed PC bottle in at 10 ^{0}C

Storage days	Sugars (mg-1d.wt.)	Starch (mg-1d.wt.)	Total protein (mg-1d.wt.)	Amino acids (mg-1d.wt.)	Total lipids (mg-1d.wt.)	Phenols (mg-1d.wt.)
0	68.54 ± 4.92	146.26 ± 6.59	85.43 ± 6.28	3.73 ± 0.08	466.08 ± 4.56	38.64 ± 2.04
1	69.16 ± 5.58	145.88 ± 7.36	83.28 ± 5.37	3.74 ± 0.06	459.56 ± 5.31	39.52 ± 3.12
2	68.77 ± 4.67	142.09 ± 6.18	82.77 ± 7.43	3.79 ± 0.08	454.79 ± 5.47	40.33 ± 3.10
3	70.38 ± 5.92	138.20 ± 5.87	79.81 ± 6.92	3.81 ± 0.12	450.87 ± 5.12	40.96 ± 4.02
4	72.33 ± 6.12	132.58 ± 6.14	76.56 ± 7.14	3.84 ± 0.14	446.38 ± 4.89	42.34 ± 3.69
5	73.17 ± 7.49	127.17 ± 5.87	74.81 ± 6.85	3.88 ± 0.16	440.62 ± 6.08	43.19 ± 4.08
6	75.62 ± 6.84	124.48 ± 5.62	71.08 ± 7.01	3.93 ± 0.22	431.05 ± 5.97	44.67 ± 3.53
7	74.11 ± 7.93	118.96 ± 6.13	69.94 ± 5.37	3.96 ± 0.24	423.86 ± 6.38	45.89 ± 4.14
8	76.25 ± 8.02	116.59 ± 5.26	68.32 ± 6.13	3.98 ± 0.20	414.55 ± 5.26	47.55 ± 3.68
10	79.36 ± 6.44	113.54 ± 6.02	64.82 ± 4.88	4.03 ± 0.18	402.64 ± 6.03	49.27 ± 2.85
15	84.59 ± 7.28	106.23 ± 5.24	63.49 ± 5.71	4.11 ± 0.24	385.87 ± 6.70	52.86 ± 3.34
20	88.26 ± 6.93	101.87 ± 6.19	62.53 ± 5.46	4.26 ± 0.18	364.48 ± 5.24	54.78 ± 4.08
25	91.77 ± 8.14	94.68 ± 5.97	60.97 ± 6.10	4.34 ± 0.24	342.14 ± 5.79	56.36 ± 3.86
30	96.13 ± 7.28	92.28 ± 5.84	58.86 ± 5.93	4.49 ± 0.22	327.83 ± 7.18	55.89 ± 4.02
60	99.53 ± 5.36	85.38 ± 4.91	57.64 ±6.15	4.84 ± 0.26	309.68 ± 7.09	57.38 ± 3.28
90	103.58± 6.98	79.56 ± 5.08	53.22 ± 4.89	5.13 ± 0.24	289.34 ± 4.13	58.03 ± 4.11
120	110.25 ± 8.19	75.21 ± 4.68	50.17 ± 3.46	5.49 ± 0.20	271.43 ± 5.04	58.59 ± 3.13
150	113.49 ± 7.31	68.54 ± 4.12	45.52 ± 5.38	5.83 ± 0.32	253.05 ± 7.68	59.79 ± 3.57
180	120.56 ± 8.72	61.08 ± 4.08	41.37 ± 4.24	6.15 ± 0.28	236.21 ± 6.83	60.11 ± 3.06

The antioxidant enzymes superoxide dismutase and peroxidase analysed during storage showed increasing trend during initial phases of storage and later a slight decrease was noticed (Table 4). The decreasing trend of these enzymes was noticed after 8 days of storage in OPL and 120 days in CPBo10℃.

Table 4: Changes in SOD and Peroxidase during seed storage in open laboratory condition in closed containers

Storage Conditions	Closed PC bottle in Laboratory Condition		Closed PC bottle in at 10 ^{0}C Condition	
Storage days	**SOD** (U/min/g of FW)	**PEROXIDASE** (U/min/g of FW)	**SOD** (U/min/g of FW)	**PEROXIDASE** (U/min/g of FW)
0	390.21 ± 2.31	62.37 ± 0.94	384.56 ± 3.74	64.78 ± 1.25
1	398.13 ± 1.92	64.28 ± 0.82	389.42 ± 3.87	66.45 ± 2.06
2	407.18 ± 2.13	68.19 ± 1.12	393.85 ± 3.91	69.07 ± 1.94
3	411.45 ± 1.73	70.43 ± 1.43	398.02 ± 4.03	71.28 ± 1.63
4	419.57 ± 2.68	73.58 ± 2.23	406.59 ± 3.78	72.85 ± 2.09
5	424.78 ± 2.34	77.91 ± 1.86	411.54 ± 4.19	74.11 ± 1.86
6	438.49 ± 1.88	81.27 ± 1.74	417.55 ± 3.84	75.66 ± 2.18
7	446.18± 2.47	83.16 ± 1.68	419.68 ± 3.66	76.18 ± 1.84
8	451.22 ± 1.82	84.17 ± 2.13	421.49 ± 3.82	79.04 ± 1.76
10	439.72 ± 2.10	76.49 ± 1.75	427.38 ± 3.44	80.93 ± 2.15
15	430.55 ± 2.41	72.92 ± 1.68	430.12 ± 5.31	82.09 ± 1.89
20	418.09 ± 2.49	74.20 ± 1.44	435.74 ± 4.19	85.72 ± 2.08
25			439.01 ± 4.01	86.18 ± 2.24
30			442.53 ± 3.93	87.29 ± 1.97
60			446.19 ± 3.67	89.36 ± 2.06
90			448.80 ± 3.19	90.08 ± 1.82
120			453.64 ± 3.59	90.51 ± 2.31
150			438.82 ± 4.08	82.01 ± 1.82
180			424.58 ± 3.62	71.37 ± 1.94

Discussion and Conclusion

Desiccation tolerance is one of the most important traits determining seed survival during storage and under stress conditions. However, the mechanism of seed desiccation tolerance is still unclear in detail. Storage, germination and desiccation in *M. ferrea* seeds as measured by changes in moisture levels and germination followed the pattern as of recalcitrant seeds, which cannot be dried relatively high moisture content without damaging the viability, one of the major characteristics of recalcitrant seeds (Roberts, 1973; Ellis, 1991). High moisture content is an essential requirement for recalcitrant seeds to retain viability (Purohit, *et al*., 1982; Daws, *et al*., 2006). Desiccation sensitivity of recalcitrant seeds are supposed to be major problem for long-term seed storage and conservation of genetic resources. In the present work in *M. ferrea* rapid drying of seeds kept in open laboratory condition has got rapid drying resulted the loss of MC from 53.35% to 7.39% resulted complete loss of viability and was significant at 0.01% P level (Table 1). On the other hand, seed stored in CPBo10℃ dehydrated slowly from retained MC 53.24% at 0 days to 34.68% on 180 days of storage thereby retained viability up to 150 days. In the present study, desiccation during storage resulted a series of changes in normal cellular set up where storage OPL condition the changes occurred fast and of desiccation rate was high thereby reduction in MC lead to viability loss within 20 days. Whereas at CPBo10℃ the rate of desiccation was slow and retain MC for longer period there by seeds were viable for longer period.

Increase in total soluble sugar during desiccation on storage of seeds of *M. ferrea* is a character related to the desiccation of sensitive seeds as that of tea, cocoa and jackfruit which showed decline in moisture level and viability and an increase in leachate

conductivity and soluble carbohydrate levels (Chandel, *et al*., 1995) in *Hydnocarpus alpinia* (Kamarudeen, *et al*., 2015), and in *Hopea ponga* (Mithun, *et al*., 2020b). While starch content was decreased during desiccation in line with the observation of Bewely and Black, 1982 and this reduction in starch content may be the reason for the increase in TSS which control the osmoticum of the cells to retain viability. Steadman, *et al*., 1996 reported that sucrose may play a protective role in plant tissue desiccation tolerance, but is not in itself sufficient to confer survival of dehydration.

Reduction in total protein content during storage desiccation in *M. ferrea* seeds resulted in the increase of free amino acids. This was in conformity with the results of Finch-savage, *et al*., 1996 and Chaithanya, *et al*., 2000. Seeds stored in OPL condition denaturation of total protein and subsequent production free amino acid was faster than in seeds stored in CPBo10℃ thereby short viability in former and extended viability in the latter case. The decrease in protein levels which resulted in the formation of free amino acids which proceeded towards non-viable seeds was reported by Bewley and Black, 1982.

In the present study in *M. ferrea* seeds levels of lipids decreased with storage and desiccation is in confirmation with the studies of Stewart and Bewley, 1980 and Bewley and Black, 1994. There are reports on the increase of fatty acids with the progress of deterioration of seeds and the role of lipids in membrane structure have led some workers to suggest that viability of seeds is closely associated with membrane integrity. So, the present investigation on lipid analysis gave little insight though the hydrophobic nature of lipids may be a factor in cell trauma (Connor, *et al*., 2001).

Active oxygen species (AOS) are involved in various aspects of seed physiology. Their generation, which occurs during seed desiccation, germination and ageing, may lead to oxidative stress and cellular damage, resulting in seed deterioration. However, cells are endowed with detoxifying enzymes and antioxidant compounds that scavenge AOS and participate in seed survival. The detoxifying mechanisms play a key role in acquisition of desiccation tolerance of developing seeds, completion of seed germination and seed storability. Activity of enzymatic antioxidants *viz*., POD, SOD, CAT, and APX are up regulated as an antioxidant defence system against endogenous oxidant radicals generated during seed germination, desiccation, maturation, storage and ageing. In *M. ferrea* seed during storage recorded an increase in the total phenol content with advancement of storage indicate that phenolics that have the antioxidant capability because of redox properties can neutralize or quench the highly reactive hydroxyl radical (OH), singlet and triplet oxygen (Dona, *et al*., 2013). Studies on seed deterioration substantiate that the fluctuations in weather conditions, *i.e.* biotic and abiotic factors contribute reactive oxygen species (ROS) production (Yadav, *et al*., 2006; Sano, *et al*., 2016). These radicals have high reactivity and damage lipids, DNA, RNA and proteins that ultimately lead to cell death (Kumar, *et al*., 2015 and 2016). The role of antioxidant enzymes like PO and SOD enzymatic antioxidants are an important class of phyto-chemicals that plays a very crucial role in maintaining seed quality by counteracting the oxidative stress (Kartoori, *et al*., 2018). Activity of enzymatic antioxidants *viz*., POD, SOD, bis up regulated as an antioxidant defence system against endogenous oxidant radicals that may occur during seed maturation, desiccation, storage, germination and ageing. In the present study in *M. ferrea* seed the increased level of total phenol content and activities of PO and SOD helped to retain viability of seeds for longer period in the storage in CPBo10℃.

Acknowledgements

Authors acknowledge Kerala State Council for Science Technology and Environment for the financial assistance as Emeritus Fellowship to PNK and Director KSCSTE-MBGIPS for facilities and encouragements.

References

1. Anonymous. "The Wealth of India." *CSIR, New Delhi, India* (1952): 3-141.
2. Arunachalam, A., Khan, M.L. and Singh, N.D. "Germination, growth and biomass accumulation as influenced by seed size in *Mesua ferrea* L." *Turkish Journal of Botany* 27 (2003): 343–348.
3. Berjak, P., Pammenter, N.W. and Christina, V. "Hydrogenous (recalcitrant) seeds: developmental status, desiccation sensitivity and the state of water in axes of *Landolphia kirkii* Dyer." *Planta* 186(2) (1992): 249-261.
4. Berjak, P., Christina, W.V. and Pammenter, N.W. "Effects of developmental status and dehydration rate on characteristics of water and desiccation-sensitivity in recalcitrant seeds of *Camellia sinensis*." *Seed Science Research* 3 (1993): 155–166.
5. Bewley, J.D. and Michael, B. "Seeds: Physiology of development and germination." (2nd edition) *New York, Plenum Press* (1994).
6. Bligh, E.G. and Dyer, W.J. "A rapid method of total lipid extraction and purification." *Canadian journal of biochemistry and physiology* 37.8 (1959): 911-917.
7. Chaitanya, K.K., S. Keshavkant. and Naithani, S.C. "Changes in total protein and protease activity in dehydrating recalcitrant Sal (*Shorea robusta*) seeds." *Silva Fennica* 34.1 (2000): 71-77.
8. Chanda, S., Kalpna, R. and Jigna, P. "Indian medicinal herb: antimicrobial efficacy of *Mesua ferrea* L. seed extracted in different solvents against infection causing pathogenic strains." *Journal of Acute Disease* 2.4 (2013): 277-281.
9. Chandel, K.P.S., Rekha, C., J. Radhamani. and Mallik, S.K. "Desiccation and freezing sensitivity n recalcitrant seeds of Tea, Cocoa and Jack fruit." *Annals of Botany* 76.5 (1995): 443-450.
10. Singh, C., Boraiah, K.M., Rajiv, K. S., Kumar, S.J., Gyanendra, S. and Ramesh, C. "Comparative study of floral biology using detergent and confirm self-incompatibility system in protogynous lines of Indian mustard (Brassica juncea)." *Indian Journal of Agricultural Sciences* 88.8 (2018): 1190–7.
11. Connor, K.F., Frank, T. B., Jack, A. V. and Kossman-Ferraz, I. "Changes in desiccating seeds of temperate and tropical forest tree species." *Journal of Sustainable Forestry* 10.3/4 (2000): 319-326.
12. Daws, M.I., Nancy, C.G. and Pritchard, H.W. "Prediction of desiccation sensitivity in seeds of woody species, a probabilistic model based on two seed traits and 104 species." *Annals of Botany* 97.4 (2006): 667-674.
13. Dona, M., Alma, B., Andrea, M., Graziano, R., L. Ventura., A. Buttafava., Anca, M., Sabatini, M. E.. A. Valassi. and Daniela, C. "DNA profiling, telomere analysis and antioxidant properties as tools for monitoring *ex-situ* seed longevity." *Annals of Botany* 111.5 (2013): 987–998.
14. Ellis, R.H. "The longevity of seeds." *Horticulture Science* 26.9 (1991): 1119-1125.
15. Farrant, J.M., P. Berjak. and Pammenter, N.W. "The effect of drying rate on viability retention of recalcitrant propagules of *Avicennia marina*." *South African Journal of Botany* 51.6 (1985): 432-438.
16. Farrant, J.M., Pammenter, N.W. and Patricia, B. "The increasing desiccation sensitivity of recalcitrant *Avicennia marina* seeds with storage time." Physiologia Plantarum 67 (1986): 291-298.
17. Farrant, J.M., Pammentern, N.W. and Patricia, B. "Recalcitrance - a current assessment." *Seed Science and Technology* 16 (1988): 155-166.
18. Farrant, J.M., Pammentern, N.W. and Patricia, B. "Germination-associated events and the desiccation sensitivity of recalcitrant seeds - a study on three unrelated species." *Planta* 178 (1989): 189–198.
19. Finch-Savage, W.E., Blake, P.S. and Clay, H.A. "Desiccation stress in recalcitrant *Quercus robur* L. seeds results in lipid peroxidation and increased synthesis of jasmonates and abscisic acid." *Journal of Experimental Botany* 47.5 (1996): 661-667.
20. Hong, T.D. and Ellis, R.H. "A comparison of maturation drying, germination, and desiccation tolerance between developing seeds of *Acer psuedoplatanus* L. and *Acer platenoides* L." *New Phytologist* 116 (1990): 589-596.

21. ISTA. "International rules for seed testing 2015." *International Seed Testing Association, Bassersdorf, Switzerland* (2015).
22. Kamarudeen, M., Babu, K.P., Jee, G., Anilkumar, C., Pandurangan, A.G., Krishnan, P.N. "Effect of desiccation on germination and biochemistry in seeds of *Hydnocarpus alpinia* Wight, an endangered medicinal tree species of Southern Western Ghats India." *Journal of Advances in Biological Sciences* 2.1&2 (2015): 7-12.
23. Kar, A. and Jain, S.R. "Investigations on the antibacterial activity of some Indian indigenous aromatic plants." *Flavour industry* 2(197): 111-3.
24. Saisanthosh, K., Sumalatha, G.M., Shuba, A.C., Komala, N.T. and Biradar Patil, N.K. "Role of enzymatic antioxidants defense system in seeds." I*nternational Journal of Current Microbiology and Applied Sciences* 7.1 (2018): 584-594.
25. Khan, M.L., Bhuyan, P., Singh, N.D. and Todaria, N.P. "Fruit set, seed germination and seedling growth of *Mesua ferrea* L. in relation to light intensity." *Journal Tropical Forest Science* 14 (2002): 35-48.
26. King, M.W. and Roberts, E.H "Maintenance of recalcitrant seeds in storage." *Recalcitrant crop seeds. Kuala Lumpur, Tropical Press SDN.BDH* (1980): 53–89.
27. Kumar, A., Dinesh, K.A., Sunil, K., Reddy, Y. M., Anjani, D.C., Saritha, K.V., Govind, P. and Kumar, S.J. "Nutraceuticals derived from seed storage proteins: Implications for health wellness." *Biocatalysis and Agricultural Biotechnology* 17(2019): 710-719.
28. Lowry, O.H., Rosbrough, N.J., Farr, A.L. and Randall, R.J. "Protein measurement with Folin- phenol reagent." *The Journal of Biological Chemistry* 193 (1951): 265-271.
29. Mazumder, R., Sujata, G.D., Basu, S.P., Avijit, M. and Singh, S.K. "Antibacterial potentiality of *Mesua ferrea* Linn. flowers." Phytotherapy Research: *An international journal devoted to pharmacological and toxicological evaluation of natural product derivatives*. Oct; 18.10 (2004): 824-6.
30. Mc Cready, R.M., Guggolz, J., Siliera, V., Owens, H.S. "Determination of starch and amylase in vegetables." *Analytical Chemistry* 22 (1950): 1156-1158.
31. Mithun, V., Babu, K.P., Pradeep, N.S. and Krishnan, P.N. "Phenology and seed development in *Mesua ferrea* L., a rare medicinal tropical tree species." *Tropical Plant Research* 7.2 (2020a): 406-414.
32. Montgomery, R. "Determination of glycogen." *Archives of Biochemistry and Biophysics* 67 (1957): 378-386.
33. Mithun, V., Pradeep, N.S. and Krishnan, P.N. "Physiological and biochemical changes during seed development in Hopea ponga (Dennst.) Mabberley: An endemic endangered tree species of Western Ghats. *Journal of Plant Science and Research* 7(2020b): 193
34. Pritchard, H.W. "Water potential and embryonic axis viability in recalcitrant seeds of *Quercus rubra*." *Annals of Botany* 67(1991): 43–49.
35. Purohit, A.N., Sharma, M.M. and Thapliyal, R.C. "Effect of storage temperature on the viability of Sal and Talura seeds." *Forest Science* 28 (1982): 526-539.
36. Roberts, E.H. "Predicting the storage life of seeds." *Seed Science and Technology* 1 (1973): 499–514.
37. Sadasivam, S. and Manickam, A. "Biochemical Methods, 2nd edition." *New Age International Publishers. New Delhi, India* (1996): 256.
38. Sano, N., Loic, R., Helen, M. N., Isabelle, D., Annie, M.P. and Mitsunori, S. "Staying alive: molecular aspects of seed longevity." *Plant and Cell Physiology* 57.4 (2015): 660-674.
39. Sokal, R.R. and Rohlf, F.J." Biometry, New York: WH Freeman and Co, 2nd ed." 584.
40. Steadman, K.J., Pritchard, H.W. and Dey, P.M. "Tissue-specific soluble sugars in seeds as indicators of storage category." *Annals of Botany* 77 (1981): 667-674.
41. Stewart, R.R.C. and Bewely, J.D. "Lipid production associated with accelerated aging of soybean axes." *Plant Physiology* 65(1980): 245-248.
42. Swain, T. and Hillis, W.E. "The phenolic constituents of *Prunus domestica*, the quantitative analysis of phenolic constituents." *Journal Science Food and Agriculture* 10(1959): 63–68.

43. Tompsett, P.B. and Pritchard, H.W. "Water status changes during development in relation to the germination and desiccation tolerance of *Aesculus hippocastanum* L. seeds." *Annals of Botany* 71(1993): 107–116.
44. Yadav, S., Bhatia, V.S. and Guruprasad, K.N. "Oxyradical accumulation and rapid deterioration of soybean seeds due to field weathering." *Indian Journal of Biochemistry and Biophysics* 43(2006): 41–47.
45. Zheng, M., Hussain, S., Tao, Y., Jiang, Q., Peng, S. and Huang, J. "Seed priming in dry seeded rice: consequences for emergence, seedling growth and associated metabolic events under drought stress." *Plant Growth Regulation.* 78 (2015): 167–178.

Source of support: Nil;
Conflict of interest: The authors declare no conflict of interests.

Cite this article as:
Mithun, V., Pradeep, N.S. & Krishnan, P.N. "Biochemical Changes during the Seed Storage of *Mesua ferrea* L., A Medicinal Tropical Tree Species." *Annals of Plant Sciences*.12.03 (2023): pp. 5773-5782.