Biological Forum – An International Journal **15(4): 155-161(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Extension of Seed Longevity of *Myristica malabarica* Lam. by Optimizing Storage conditions

Suresh Kumar K.A.[1*], Anilkumar C.[2], Ajithkumar K.G.[3], Prajith T.M.[4] and Amritha V.V.[5]
[1]Assistant Professor, Department of Botany,
Government College Chittur, Palakkad (Kerala), India.
[2]Head, Conservation Biology Division, Jawaharlal Nehru Tropical Botanic Garden and Research Institute,
Pacha-Palode, Thiruvananthapuram (Kerala), India.
[3]Associate Professor, Department of Botany,
Government College for Women, Thiruvananthapuram (Kerala), India.
[4]Assistant Professor, Department of Botany,
Sree Krishna College, Guruvayur, Thrissur (Kerala), India.
[5]Ph.D. Scholar, Department of Botany,
Government College for Women, Thiruvananthapuram, (Kerala), India.

(Corresponding author: Suresh Kumar K.A.)*
(Received: 20 February 2023; Revised: 05 March 2023; Accepted: 16 March 2023; Published: 20 April 2023)
(Published by Research Trend)

ABSTRACT: ***Myristica malabarica*** **is an evergreen arborescent species of the family** ***Myristicaceae*****. As a major floral component of a unique but degrading ecosystem in the Western Ghats called** ***Myristica*** **swamp, this species is enlisted by IUCN as vulnerable. The seeds of** ***M. malabarica*** **are recalcitrant in nature and lost its viability within a few days of shed in normal conditions. The sensitivity of the seeds to desiccation and lower temperature imposes constraints in seeds storage for longer period. In the present study, the fresh seeds of** ***M. malabarica*** **were stored in three treatments (T) with different temperatures and relative humidity (RH) such as T_1 (10±2°C and 25±2 % RH), T_2 (18±2°C and 50±2 % RH) and T_3 (10±2°C and 80±2 % RH). More than 50% of seeds kept in T_1 and T_3 conditions lost viability after 15 and 30 days respectively of storage (DoS), whereas T_2 treatment was effective to extends the seed viability to 90 days. The seeds kept in open plastic trays at laboratory conditions (28 ± 2°C and 65 ± 2 % RH) was taken as control (C). Seed viability was tested by various parameters like seed germination, electrolyte conductivity, Total Dissolved Solutes (TDS), Dehydrogenase activity (DHA) and the level of total phenolic compounds and proline amino acid in the embryo. Data was analysed and presented correspond to means ± standard error. DMRT was carried out with SPSS, version 13.0 software. All treatments consisted of five replicates. The storage studies of** ***M. malabarica*** **was challenging due to their high oil content, which makes them susceptible to rancidity and fungal growth. Maintaining optimal moisture content of seed, insect and pest infestations, determination the optimal storage temperature and humidity were other major challenges during this study. The results indicate that** ***M. malabarica*** **seed viability can be prolonged for up to 90 days if they are stored under optimal conditions of 18 ± 2°C temperature and 50 ± 2 % RH.**

Keywords: *Myristica malabarica*, recalcitrant seed, Relative Humidity (RH), Leachate Conductivity (LC), Total Dissolved Solute (TDS), Dehydrogenase activity (DHA), Sterilised Distilled Water (SDW), Critical Moisture Temperature (CMC).

INTRODUCTION

The most convenient and conventional means of *ex situ* germplasm conservation is seed storage. But this method is not preferable in the case of recalcitrant seeds as they shed at high water content and the hydrated storage of seeds are constrained by germination of seeds without extraneous water and proliferation of seed associated fungi (Anilkumar *et al.*, 2002). The major challenge concerned with conservation of threatened forest trees is the seed recalcitrance and it is characterized by the inability of seeds to tolerate desiccation and lower temperature (Bates *et al.,* 1973). Proper seed storage is very important in maintaining the vigor and vitality of seeds. But if it is not possible due to situations like existence of adverse environmental condition for seed germination at the time of seed collection, transportation of seeds to distant places, long term storage of seeds for the purpose of conserving genetic resources *etc*. effective seed storage techniques will be unavoidable. *M. malabarica* Lam. is a vulnerable tree species growing in unique wet land ecosystem of Western Ghats known as *Myristica* swamps and it produces recalcitrant seeds (Berjak and Pammenter 1995). As the seeds of *M. malabarica* lose their viability within days of shed in ambient conditions (Bonner, 1996) and the constraints imposed by desiccation sensitivity, seed storage by using standard gene bank remains challenging. Considering the slow pace of its natural regeneration process and the difficulty of storage, it is absolutely essential to devise a strategy for maintaining the seed viability for a considerable period.

The misperception that recalcitrant seeds cannot be stored is based on the assumption that drying and freezing are the only storage strategy available to handle the diverse range of storage physiology observed in plant seeds. However long-term storage of recalcitrant can be achieved through development of species-specific storage conditions (Hong and Ellis 1996; Walters *et al.,* 2004; Berjak, 2006; Berjak; Pammenter 2013; Trusiak *et al*., 2022). The storability of recalcitrant seeds can be prolonged when they are kept under refrigerated conditions, but once they are exposed to critical moisture levels, they become capable of germinating (Wen, 2009; Devi *et al.,* 2016; Nagendra *et al.,* 2019). Prolonging of storage period of recalcitrant seed is possible by regulating the movement of water (Cecel and Barbedo 2023) and optimizing the storage temperature (Bareke *et al.*, 2022).

MATERIALS AND METHODS

Seed source and surface sterilization. Mature fruits of *M. malabarica* were collected from the *Myristica* swamp forests, Kulathupuzha Forest Range, (8°45'82" N and 77°10'55"E) Kollam District, Kerala, India, which is situated at an elevation of 155 meters from the sea level. The collected fruits were brought to the laboratory in sealed polythene bags. The seeds were removed from the fruits and dearillated. Seeds did not have any apparent physical damage or infestation by insects were selected the seed surface was sterilized with 1% sodium hypochlorite for 30 minutes and washed thoroughly with sterilized distilled water for five times. The processed seeds were used for germination and storage studies.

Seed storage trial. The evaluation of the storage potential of the seeds were done by keeping the seeds under the following laboratory conditions. For each test, 5 replicates of samples were used and each replica consists of 50 seeds. Seeds were taken randomly form the seed lots to study the physiological and biochemical aspects at various stages of storage.

1. Mature seeds with moisture content 31.48 ± 0.16 %were kept in open plastic trays placed inside the poly tunnels at laboratory conditions (28 ± 2°C and 65±2% RH) which was taken as control (C). The temperature and humidity in the poly tunnels were regularly monitored by using thermometer and hygrometer. The ambient conditions were maintained stable by misting of water.

2. Mature seeds with shedding were stored in polythene bags separately at various storage conditions like lowered temperature and humidity (10±2°C and 25±2 % RH) as Treatment 1 (T_1); moderate temperature and humidity (18±2°C and 50±2% RH) as Treatment 2 (T_2); lower temperature and high humidity (10 ±2°C and 80±2 % RH) as Treatment 3 (T_3). Changes in the moisture content of the seeds during different storage conditions were measured by following high constant temperature oven method on fresh weight basis (Burhan and Al-Taey 2019). Seeds kept in all storage conditions were sampled at specific intervals and seed viability was monitored by standardized seed germination tests (Harrington, 1963).

Moisture content of the seeds. Moisture content of seeds at different stages of storage were calculated by estimating the difference between weight of fresh seed and weight of seed at different storage conditions (Burhan and Al-Taey 2019). Samples were cut into small pieces and weighed before and after drying at 130 ± 20°C for 1 hour and the moisture content was calculated as the percentage of water on fresh weight basis.

Seed Viability. The viability of the seeds at different stages of storage were assessed by Dehydrogenase activity (DHA) test (ISTA, 2008). Each DHA test was conducted with five replicates. Each seed was preconditioned by soaking 4 hours (hrs.) in SDW. The embryos were taken out and incubated for 24 hrs in 3 ml of 1 % 2, 3, 5 - triphenyl tetrazolium chloride (TTZ). The stain intensity on the embryo was assessed visually and recorded the colour variations. Stained embryonic tissues were then soaked individually in 5ml of methyl cello solve solution for 4 hrs. The extract was decanted and the colour intensity was assessed by using spectrophotometer (UV - VIS spectrophotometer, Shimadzu UV - 1800) at 480 nm.

The variation in Leachate Conductivity (LC) or electrolyte conductivity and Total Dissolved Solutes (TDS) were also taken as parameters to analyse the seed viability. Electrolyte conductivity of mature seeds at different stages of storage were measured according to the method followed by (ISTA, 2008). One seed from each of the five replicates with equal weight from all seed lots were incubated in 40 ml of deionized distilled water and kept in a closed container for 24 hrs. at 28 ± 2°C in the laboratory. Care was taken to immerse the seeds completely in the deionized distilled water. The conductivity of the solution was measured with conductivity meter (Systronics, DDR, type 306) and expressed as micro-Siemens (μS/cm) (IUCN, 2020).

Total Dissolved Solutes (TDS) of mature seeds at different storage conditions were analysed by following the same method used to measure electrolyte conductivity. One seed from each of the five replicates with equal weight from all seed lots were incubated in 40 ml of deionized distilled water which was sufficient to sink the seeds completely and kept in a closed container for 24 hrs at 28 ± 2°C in the laboratory The Total Dissolved Solid in the solution was measured with a conductivity meter (Systronics, Conductivity TDS meter 308) and expressed as parts per million (ppm).

Estimation of total phenolics and proline. The estimation of total phenolics in the embryowas done by following the method (Kamarudeenkunju, 2003). Proline content in the embryonic axis was estimated by the method (Khajjak *et al*., 2016).

Seed germination test. Germination potential of seeds at different stages of storage were assessed by conducting germination test under standardized conditions. Seed viability was determined on the basis of the percentage of seeds germinated. The seeds were considered as germinated while the radicle emerged out to the seed coat at length of 5 mm (Harrington, 1963). Germination tests were done in five replicates of 50 seeds each. Seeds from different storage conditions were wrapped with wet acid free germination paper and kept it in a seed germinator (KEMI SEED GERMINATOR KSG-2) without light (30 ± 2°C, 80± 2 % RH).

Statistical analysis of data. Data presented correspond to means ± standard error. DMRT was carried out with SPSS, version 13.0 software. All treatments consisted of five replicates.

RESULTS AND DISCUSSION

As the seed viability has been drastically declined after desiccation of the seeds to a certain limit, seeds of *Myristica malabarica* is catagorised as recalcitrant seeds (Bonner, 1996). Storage trials showed that seed viability was mainly determined by temperature and relative humidity of the storage system. Like the other recalcitrant seeds like *Saracaasoca* (Kittock *et al*., 1968; Meryman, 1968) *Madhuca indica* (Parvathy *et.al*., 2020), *M. malabarica* seeds also showed sensitivity towards freezing temperature and desiccation.

The seeds stored at open laboratory condition (C) have registered maximum germination 93.2 ±1.74%) when the seed moisture content was 31.48 ± 0.16%. The seed germination rate was declined gradually and significantly ($p < 0.01$) as the seed desiccation proceeds. But sharp declination of seed germination from 67.2 ± 0.49% to 19.2 ± 0.49 % was registered when the seed moisture content was reduced from 14.32 ± 0.15 % to 12.18 ± 0.11 %. The seed viability has been lost fully at which the seed moisture level was 8.28 ± 0.16 % (Table 1). The embryo of the viable seeds was stained deeply and observed as deep red in colour when incubated in tetrazolium chloride (TTZ) solution and the absorbance of formazan which is the indicator of the intensity of the TTZ absorbed by embryonic tissue was very high compared to that of nonviable seeds which were stained feebly or unstained. Leachate conductivity and TDS of the viable seeds were 5 and 3 times respectively lesser than that of nonviable seeds. The level of total phenolic compounds and proline amino acid in the embryonic tissue under all the storage conditions were increased as the desiccation induced stress proceeds (Table 2 a,b,c). The viability of the seeds has been completely lost by 30 days and 45 days respectively while the seeds were stored in T_1 and T_3 condition. But under the storage condition T_2, the seed viability was maintained up to 90 days (Table 2b).

The drastic decline of seed viability under control (C) from 93.2 ±1.74% to 19.2 ± 1.36 % by 8 days after storage at open laboratory condition can be attributed to lowered moisture content in the embryo. Report are that lowered water contents for prolonged period may eventually leads to accumulation of aqueous based damages includes mechanical damage to cell membrane system (Oaikhena *et al*., 2013) and macromolecular denaturation (Pammenter and Berja 2000). A sharp declination seed germination when the seed moisture content is below 14.32 ± 0.15% implies that it is the critical moisture content (CMC) of *M. malabarica* seed can be attributed to elevated level of ABA (Gayatri *et al.,* 2022). The detrimental effect of dehydration below the critical moisture content, on membrane system and biomolecules in the cell has also been reported in many recalcitrant seeds (Pillai and Pandalai 2015; Prajith and Anilkumar 2017; Fazeli-Nasab *et al.,* 2022).

The seed storage at lower temperature and relative humidity (T_1) as well as at lower temperature and higher relative humidity (T_3) have not shown positive response in prolonging storage life of seeds (Table 2a and 2c) due to the intolerance of recalcitrant seeds to lower temperature and desiccation (Roberts, 1972; Szuba *et al.,* 2022). The intolerance to lower temperature is due to the formation of large ice crystals intracellular space (Roberts, 1973). and structural deformation of vital macromolecules and membrane system in the cells (Shamsual Hayat *et al.,* 2012).

The seeds stored under T_2 could retain moisture content above the Critical Moisture Content (CMC) and are maintained seed viability up to 90DoS (Table 2b). The extension of seed viability period under T_2 treatment was explained by the fact that under moderate RH and temperature, the biological process in the seed did not cease but only slowed (Sharanya *et al*., 2022). The analysis of the physiological parameters like TTZ stainability, level of total phenolics and proline amino acid of embryonic tissue, electrolyte leakage and total dissolved solids (TDS) of the seed also showed that seed moisture content has a vital role in retaining the viability of the seeds of *M. malabarica.* The tissues from the embryo of viable seed which were incubated in tetrazolium chloride (TTZ) solution for 24 hrs. has stained deeply and appeared deep red (DR) in colour. The stainability of the embryonic tissue has been changed gradually from deep red (DR) to red (R), pale red (PR) and unstained (US) as the moisture content of the seed was reduced and so also the viability. The absorbance of formazan which is the indicator of the intensity of the TTZ absorbed by embryonic tissue and seed viability was high in viable seeds and very low in non-viable seeds (Table 1, 2a, 2b and 2c) (Smitha and Das 2016). as in *Hopea parviflora* and *Vateria indica* (Swain and Hillis 1959). Seed dehydration directly influenced the drop of tripheny formazan formation in embryonic tissues of *M. malabarica*, which can be attributed to the lowered activity of dehydrogenase enzymes and it inferred the shift of cell from living to non - respiring dead cells (Szabados and Savoure 2010).

Fresh seeds of *M. malabarica* shows 10.31 mg/g FW of phenolic content, but in due course of different storage conditions results the significant accumulation of phenolics in seed tissues which indirectly connect with the decline of seed viability. The production of various phenolic compounds in plant tissue is mainly induced by various biotic and abiotic factors (Varghese and Naithani 2002). In T_1 condition, seed tissues where accumulates around 66.54 mg/g FW of phenolics at 15 DoS (days of storage) and the corresponding germination percentage was 19%. Among three treatments, T_2 exhibits maximum extension of viability as 62% at 90DoS, though tissues possess 91.04 mg/g FW of phenolic content. In T_3, around 50% seed viability was retained upto 30DoS and the phenolic content was 71.28 mg/g FW. Irrespective of treatments, seed tissues were unanimously significantly produced phenolic compounds in order to minimize the tissues damages.

In *M. malabarica*, the fresh seed tissues contain about 24.09µ mol/g FW of proline content, while the storage experiments induce significant proline production in tissues. Proline is an amino acid act as a compatible osmolyte (Varghese *et al.*, 2002) and it is considered as a major component of the antioxidant defense system, a regulator of cellular redox potential, a structural stabilizer of cellular organelles and macromolecules of signal transduction pathways that regulate stress - responsive genes (Verbruggen and Hermans 2008). In T_2, the seeds of 90 DoS showed 65.81µ mol/g FW of proline content while 56.34 µ mol/g FW for 30 days stored seeds of T_3. Higher level of proline been in with low water content compared to tissues with high water content (Vertucci and Farrant 1995; Walters *et al.*, 2001). The presence of phenolic compounds and proline amino acid in the embryonic tissue of T_2 were lower in concentration indicated that seeds were under least stress compared to that of control (C), T_1 and T_3 (Table 1 and 2a, 2b and 2c). The extended longevity of the seeds up to 90 DoS under T_2 storage condition can be attributed to absence of stress-free storage environment.

As the seeds stored under T_2 condition could retain the structural integrity of membrane system of the cell up to 90 DoS, electrolyte leakage and TDS recorded least value. However, as the moisture content of the seeds decreased over time, there was a gradual increase in leakage of electrolytes and other solutes from the seed tissues, which might led to a decline in seed vigor. The deterioration of the cellular membrane system of the seed might be the primary reason for the deleterious changes that occur in the seed. Similar results have been reported in *Saracaasoca* (Roxb.) W.J. de Wilde. (Meryman, 1968), *Syzygium cumini* (L.) Skeels (IUCN, 2020) and *Syzygium cumini* (L.) DC (Yadav *et al.*, 2021).

Table 1: Biochemical and physiological characteristics of seed (Embryo) in lab condition.

Treatment	DoS	Seed Germination (%)	MC (%)	TTZ	DHA	LC (µS)	TDS (ppm)	Total Phenolics (mg g^{-1} FW)	Proline (µ mol/g FW)
C (28 ± 2°C and 65 % RH)	0	93.2 ± 1.74^{a}	31.48 ± 0.16^{a}	DR	1.18 ± 0.004^{a}	10.05 ± 0.35^{a}	5.25 ± 0.30^{a}	10.31 ± 0.39^{a}	24.09 ± 1.41^{a}
	2	84.0 ± 1.10^{b}	21.36 ± 0.27^{b}	DR	1.15 ± 0.005^{a}	15.58 ± 0.79^{b}	9.27 ± 0.25^{b}	30.06 ± 1.91^{b}	13.04 ± 0.88^{b}
	4	74.8 ± 1.62^{c}	19.40 ± 0.17^{c}	DR	0.90 ± 0.006^{b}	21.39 ± 0.51^{c}	12.27 ± 0.43^{c}	60.61 ± 2.15^{c}	55.30 ± 0.56^{c}
	6	70.0 ± 0.63^{d}	17.85 ± 0.62^{d}	DR	0.72 ± 0.008^{c}	25.22 ± 0.77^{d}	15.77 ± 0.50^{d}	77.23 ± 1.58^{d}	56.94 ± 0.75^{d}
	7	67.2 ± 1.50^{e}	14.32 ± 0.15^{e}	DR	0.61 ± 0.007^{c}	29.52 ± 0.68^{e}	18.08 ± 0.43^{e}	85.16 ± 3.25^{e}	64.45 ± 0.68^{e}
	8	19.2 ± 1.36^{f}	12.18 ± 0.11^{f}	R	0.32 ± 0.008^{d}	40.81 ± 0.72^{f}	28.32 ± 0.71^{f}	101.05 ± 2.17^{f}	68.43 ± 0.89^{f}
	9	3.6 ± 1.17^{g}	9.38 ± 0.16^{g}	P R	0.18 ± 0.034^{a}	80.54 ± 0.62^{g}	55.03 ± 0.99^{g}	102.18 ± 2.16^{g}	72.43 ± 2.49^{g}
	10	00^{ns}	8.28 ± 0.16^{h}	US	0.12 ± 0.003^{e}	98.93 ± 1.11^{h}	71.29 ± 2.03^{h}	124.53 ± 1.15^{h}	95.79 ± 1.73^{h}
	11	00^{ns}	7.90 ± 0.25^{i}	US	0.11 ± 0.002^{e}	110.22 ± 3.68^{i}	93.61 ± 1.90^{i}	127.60 ± 0.96^{i}	109.51 ± 3.55^{i}

Values are Means of three replicates with Standard Error; Significant changes were indicated by letters in superscript
DoS: Days of Storage, C: Control, RH: Relative Humidity, °C: Degree Centigrade, ±: Standard Error, µS: Micro Siemen, ppm: Parts Per Million, %: Percentage, LC: Leachate Conductivity, TDS: Total Dissolved Solids, MC: Moisture Content, TTZ: 2, 3, 5 Triphenyl Tetrazolium Chloride, DR: Dark Red, R: Red, PR: Pale Red, US: Unstained, R; Red, mg: milligram, µ mol: Micromole,g: gram, FW: Fresh weight.

Table 2a: Biochemical and physiological changes of seed (Embryo) in T_1 storage conditions.

Treatment	DoS	Seed Germination (%)	MC (%)	TTZ	DHA	LC (µS)	TDS (ppm)	Total Phenolics (mg g-1 FW)	Proline (µ mol/g FW)
T_1 (10±2°C and 25 % RH)	0	93.2 ± 1.74^{a}	31.48 ± 0.162^{a}	DR	1.18 ± 0.004^{a}	10.05 ± 0.35^{a}	5.25 ± 0.30^{a}	10.31 ± 0.39^{a}	24.09 ± 1.4^{a}
	15	19.6 ± 0.75^{a}	20.26 ± 0.17^{a}	R	0.33 ± 0.023^{a}	43.77 ± 2.97^{a}	32.42 ± 1.38^{a}	66.54 ± 2.09^{a}	50.41 ± 2.1^{a}
	30	00^{ns}	12.26 ± 0.11^{a}	US	0.10 ± 0.002^{a}	96.13 ± 1.53^{a}	71.13 ± 1.33^{a}	99.26 ± 1.54^{a}	67.09 ± 2.08^{a}
	45	00^{ns}	10.24 ± 0.11^{a}	US	0.096 ± 0.003^{a}	112.5 ± 2.1^{a}	98.96 ± 2.09^{a}	117.48 ± 2.15^{a}	94.3 ± 1.48^{a}
	60	00^{ns}	9.22 ± 0.13^{a}	US	0.085 ± 0.004^{a}	127.85 ± 2.52^{a}	107.45 ± 2.46^{a}	121.18 ± 3.17^{a}	123.96 ± 0.97^{a}
	75	00^{ns}	8.30 ± 0.13^{a}	US	0.075 ± 0.002^{a}	149.93 ± 2.57^{a}	113.97 ± 2.11^{a}	128.2 ± 2.15^{a}	131.08 ± 0.65^{a}
	90	00^{ns}	8.08 ± 0.07^{a}	US	0.070 ± 0.004^{a}	166.35 ± 1.86^{a}	129.35 ± 2.64^{a}	132.9 ± 1.61^{a}	142.57 ± 0.93^{a}
	105	00^{ns}	8.02 ± 0.066^{a}	US	0.062 ± 0.002^{a}	178.78 ± 3.07^{a}	140.92 ± 0.75^{a}	138.16 ± 1.26^{a}	149.36 ± 0.95^{a}
	120	00^{ns}	7.96 ± 0.05^{a}	US	0.043 ± 0.002^{a}	191.77 ± 3.22^{a}	162.68 ± 3.64^{a}	141.57 ± 1.01^{a}	160.3 ± 0.66^{a}

Values are Means of three replicates with Standard Error; Significant changes were indicated by letters in superscript
DoS: Days of Storage, T_1: Treatment 1, RH: Relative Humidity, °C: Degree Centigrade, ±: Standard Error, µS: Micro Siemen, ppm: Parts Per Million, %: Percentage, LC: Leachate Conductivity, TDS: Total Dissolved Solids, MC: Moisture Content, TTZ: 2, 3, 5 Triphenyl Tetrazolium Chloride, DR: Dark Red, R: Red, US: Unstained, R; Red, mg: milligram, µ mol: Micromole, g: gram, FW: Fresh weight.

Table 2b: Biochemical and physiological changes of seed (Embryo) in T_2 storage conditions.

Treatment	DoS	Seed Germination (%)	MC (%)	TTZ	DHA	LC (µS)	TDS (ppm)	Total Phenolics (mg g-1 FW)	Proline (µ mol/g FW)
T_2 (18±2°C and 50 % RH)	0	93.2 ± 1.74^a	31.48 ± 0.16^a	DR	1.18 ± 0.004^a	10.05 ± 0.35^a	5.25 ± 0.30^a	10.31 ± 0.39^a	24.09 ± 1.41^a
	15	94.8 ± 0.49^b	30.87 ± 0.02^b	DR	1.16 ± 0.009^b	13.47 ± 0.60^b	8.77 ± 1.24^b	33.56 ± 1.51^b	20.61 ± 0.61^b
	30	94.4 ± 0.75^a	30.24 ± 0.03^b	DR	$0.90 \pm .005^b$	17.84 ± 1.46^b	11.7 ± 1.38^b	35.41 ± 0.75^b	21.43 ± 0.55^b
	45	86.8 ± 0.49^a	30.00 ± 0.06^b	DR	0.72 ± 0.007^b	24.41 ± 1.69^b	13.47 ± 1.34^b	51.37 ± 0.39^b	31.15 ± 0.38^b
	60	70.8 ± 1.02^a	29.94 ± 0.05^b	DR	$0.61 \pm .007^b$	25.03 ± 1.12^b	15.66 ± 1.47^b	58.47 ± 1.50^b	39.10 ± 0.69^b
	75	64.8 ± 1.02^a	29.86 ± 0.09^b	R	0.32 ± 0.004^b	35.43 ± 1.87^b	20.88 ± 1.70^b	70.25 ± 1.46^b	54.54 ± 1.28^b
	90	$62.4 \pm .075^a$	29.57 ± 0.17^b	US	0.18 ± 0.024^b	36.25 ± 1.54^b	21.44 ± 1.06^b	91.04 ± 1.14^b	65.81 ± 1.86^b
	105	18.4 ± 0.75^a	27.14 ± 0.16^b	US	0.12 ± 0.002^b	45.56 ± 1.59^b	27.56 ± 1.93^b	129.78 ± 1.77^b	82.54 ± 2.34^b
	120	00^{ns}	26.14 ± 0.19^b	US	$0.11 \pm .002^b$	113.34 ± 1.27^b	88.88 ± 1.89^b	134.32 ± 2.84^a	90.18 ± 3.06^b

Values are Means of three replicates with Standard Error; Significant changes were indicated by letters in superscript

DoS: Days of Storage, T_2: Treatment 2, RH: Relative Humidity, °C: Degree Centigrade, ±: Standard Error, µS: Micro Siemen, ppm: Parts Per Million, %: Percentage, LC: Leachate Conductivity, TDS: Total Dissolved Solids, MC: Moisture Content, TTZ: 2, 3, 5 Triphenyl Tetrazolium Chloride, DR: Dark Red, R: Red, US: Unstained, R; Red, mg: milligram, µ mol: Micromole, g: gram, FW: Fresh weight.

Table 2c: Seed Biochemical and physiological changes of seed (Embryo) in T_3 storage conditions.

s	DoS	Seed Germination (%)	MC (%)	TTZ	DHA	LC (µS)	TDS (ppm)	Total Phenolics (mg g-1 FW)	Proline (µ mol/g FW)
T_3 (10±2°C and 80 % RH)	0	93.2 ± 1.74^a	31.48 ± 0.16^a	DR	1.18 ± 0.004^a	10.05 ± 0.35^a	5.25 ± 0.30^a	10.31 ± 0.39^a	24.09 ± 1.41^a
	15	70.0 ± 1.30^c	31.42 ± 0.14^b	R	1.13 ± 0.011^b	25.49 ± 1.88^c	14.48 ± 1.19^c	59.32 ± 0.97^c	37.88 ± 0.91^c
	30	50.4 ± 0.75^b	30.38 ± 0.09^b	R	0.885 ± 0.005^c	38.4 ± 1.39^c	19.84 ± 1.12^c	71.28 ± 0.7^c	56.34 ± 0.86^c
	45	21.6 ± 0.75^b	29.24 ± 0.10^c	US	0.699 ± 0.004^c	40.50 ± 1.91^c	22.51 ± 1.07^c	103.8 ± 1.47^c	71.75 ± 1.81^c
	60	14.0 ± 1.67^b	29.18 ± 0.08^c	US	0.599 ± 0.008^b	58.12 ± 1.97^c	30.48 ± 1.11^c	115.67 ± 2.87^a	75.30 ± 2.11^c
	75	06.8 ± 0.8^b	29.16 ± 0.14^c	US	0.313 ± 0.005^b	67.2 ± 1.33^c	38.41 ± 1.07^c	120.08 ± 3.5^a	82.21 ± 2.75^c
	90	5.2 ± 0.49^b	28.68 ± 0.08^c	US	0.169 ± 0.021^b	72.96 ± 1.33^c	40.86 ± 1.59^c	129.35 ± 2.64^a	84.88 ± 1.90^c
	105	00^{ns}	27.22 ± 0.49^b	US	0.109 ± 0.004^b	122.24 ± 2.53^c	105.06 ± 1.78^c	131.4 ± 1.81^b	86.64 ± 2.21^b
	120	00^{ns}	27.02 ± 0.52^b	US	0.102 ± 0.0004^c	162.93 ± 1.79^c	124.95 ± 1.66^c	140.91 ± 3.35^a	89.42 ± 2.18^b

Values are Means of three replicates with Standard Error; Significant changes were indicated by letters in superscript.

DoS: Days of Storage, T_3: Treatment 3, RH: Relative Humidity, °C: Degree Centigrade, ±: Standard Error, µS: Micro Siemen, ppm: Parts Per Million, %: Percentage, LC: Leachate Conductivity, TDS: Total Dissolved Solids, MC: Moisture Content, TTZ: 2, 3, 5 Triphenyl Tetrazolium Chloride, DR: Dark Red, R: Red, US: Unstained, R; Red, mg: milligram, µ mol: Micromole, g: gram, FW: Fresh weight.

CONCLUSIONS

The main propagation material of *M. malabarica* is the seed. However, the sensitivity of recalcitrant seeds towards desiccation and freezing temperature while storing poses a major limitation to germplasm conservation. To overcome this hurdle, the seeds of *M. malabarica* can be stored under specific conditions of 18 ± 2°C and 50 ± 2 % RH. This recommended storage condition not only minimizes the limitations of seed storage but also proves to be a cost-effective method for the germplasm preservation of *M. malabarica*, thereby extending its seed longevity.

FUTURE SCOPE

The optimized storage condition identified in this study looks promising to improve the longevity and viability of recalcitrant seeds which are highly sensitive to desiccation and have a short storage life.

Acknowledgement. The authors greatly acknowledge Department of Forest, Government of Kerala for permitting the seeds from the *Myristica* swamp forests, Kulathupuzha Forest Range, (8°45′82″N and 77°10′55″E) Kollam District, Kerala, India. We also acknowledge Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Palode, Thiruvananthapuram, Kerala for providing laboratory facilities to do the biochemical analysis of the seed.

Conflict of interest. None.

REFERENCES

Anilkumar, C., Babu, K. P. and Krishnan, P. N. (2002). Seed storage and viability of *Myristica malabarica* Lam. an endemic species of Southern Western Ghats (India). *Seed Science and Technology*, *30*, 651- 657.

Bareke, T., Addi, A., Roba, K. and Kumsa, T. (2022). Effect of storage temperature and packing materials on seed germination and seed storage behavior of Schefflera abyssinica. *Nusantara Bioscience*, *14*(2).

Bates, L. S., Waldeen, R. P. and Teare, I. D. (1973). Rapid determination of free proline for water- stress studies. *Plant and Soil*, *39*, 205 – 207.

Berjak, P. and Pammenter, N. W. (2013). From Avicennia to Zizania: seed recalcitrance in perspective. *Annals of botany, 111*(2), 213-228.

Berjak, P. (2006). Unifying perspectives of some mechanisms basic to desiccation tolerance across life forms. *Seed Science Research, 16*(1), 1-15.

Berjak, P. and Pammenter, N. W. (1995). Recalcitrant (desiccation - sensitive) seeds. In Innovations in tropical tree seed technology (eds, K. Olesen), Proceedings, IUFRO Symposium of the Project Group P.2.04.00, Seed Problems; 1995 September 7 - 10; Arusha, Tanzania, Copenhagen, Denmark: National Tree Seed Programme, 14 - 29.

Bonner, F. T. (1996). Responses to drying of recalcitrant seeds of *Quercus nigra* L. *Annals of Botany*, *78,* 181-187.

Burhan A. K. and AL-Taey D. K. A. (2019). Effect of Potassium humate, humic acid and compost of rice wastes in the growth and yield of two cultivars of Dill under salt stress conditions. *Advances In Natural and Applied Sciences*, *12*(11), 1-6.

Cecel, A. T. and Barbedo, C. J. (2023). Storage of recalcitrant seeds of *Eugenia brasiliensis* Lam. under control of water availability. *Journal of Seed Science, 45*, e202345009.

Devi, C A, Swamy, G. S. K. and Nagesh, N. (2016). Studies on storage and viability of jamun seeds (*Syzygium cuminii* L. Skeels). *Bioscience Biotechnology Research Asia, 13*(4), 2371-2378.

Fazeli-Nasab, B., Jami, R., Vahabi, N., Rezaei, A., Piri, R. and Valizadeh, M. (2022). Desiccation Tolerance in Orthodox and Recalcitrant Seeds. In *Plant-Microbe Interactions* (pp. 265-282). CRC Press.

Gayatri, G. P., Kumar, K. A., Nair, P. S., Deth, G. S. K. and Baiju, K. V. (2022). Dynamics of water and abscisic acid during embryogeny and embryo drying in the recalcitrant seeds of *Vateria indica* L. *Journal of Plant Growth Regulation*, *41*(1), 15-22.

Harrington, J. F. (1963). Practical advice and instructions on seed storage. *Proceedings of International Seed Testing Association, 28*, 989-994.

Hong, T. D. and Ellis, R. H. (1996). A protocol to determine seed storage behaviour. *IPGRI Technical Bulletin*, 1-27.

ISTA (2008). International Rule for Seed testing association, News Bulletin, 135. *Seed Science and Technology*, 31 - 33. (ISTA News Bulletin No. 135 April 2008. p: 31 - 33).

ISTA (2008). International rules for seed testing, Rules. Annexe to chapter 5, *Seed Science and Technology 21*, Supplement, 141 - 186.

IUCN (2020). AMP Workshops on Medicinal Plants, India (January 1997). 1998. *Myristica malabarica*. The IUCN Red List of Threatened Species 1998: 2020, e.T31219A9615327.

Kamarudeenkunju (2003). Physiological and biochemical studies on seeds of *Hopea parviflora* Bedd. and *Vateria indica* Linn.: two economically important endemic trees of western Ghats. Ph. D Thesis, Kerala University, Thiruvananthapuram.

Khajjak, M. H., Raza, A. M., Luqman, M., Yusafzai, A., Naudhani, S., Ali, G. M. and Saeed, M. (2016). Evaluation of seed viability of *Juniperus excelsa* (M. Bieb.) found in Balochistan by Tetrazolium assay. *Journal of Applied and Emerging Sciences*, *6*(1), 31-35.

Kittock, D. L. and Law, A. G. (1968). Relationship of seedling vigour, respiration and tetrazolium chloride reduction by germinating wheat seeds. *Agronomy Journal*, *60,* 286-288.

Meryman, H. T. (1968). Modified model for the mechanism of freezing injury in erythrocytes. *Nature*, *218*, 333–336.

Nagendra, M. S., Tamilarasan, C., Shobha, K. V., Ragupathi, K. P. and Renganayaki, P. R. (2019). Refrigerated Storage of Recalcitrant Seeds Reduces the Seed Viability: A Myth? *Research Journal of Agricultural Sciences, 10*(2), 455-456.

Oaikhena, E. E., Ajibade, G. A., Appah, J. and Bello, M. (2013). Dehydrogenase enzyme activities in germinating cowpea *Vigna unguiculata* (L) Walp. *Journal of Biology, Agriculture and Healthcare*, *3,* 32-36.

Pammenter, N. W. and Berjak, P. (2000). Aspects of recalcitrant seeds physiology. *Brazilian Journal of Plant Physiology*, *12*, 56 - 69.

Parvathy, S. Nair., Ajith Kuma,r K. G., Gayathri, G. P. and Anil Kesava Dath G. (2020). Recalcitrant behavior of seeds of *Syzygium cumini* (L.) Skeels during embryogeny and natural desiccation. *Plant Physiology Reports, 25*, 426-131.

Pillai P. K. C. and Pandalai, R. C. (2015). Storage practices in recalcitrant tropical forest seeds of Western Ghats. KFRI Research Report No. 496: 2015, 33pp.

Prajith, T. M. and Anilkumar, C. (2017). Seed desiccation responses in *Saraca asoca* (Roxb.) W.J. de Wilde. *Current Science*, *12,* 2462–2466.

Roberts, E. H. (1972). Viability of seeds. London, Chapman and Hall.

Roberts, E. H. (1973). Predicting the storage life of seeds. *Seed Science and Technology*, *1,* 499-514.

Shamsual Hayat, Qaiser Hayat, Mohammed Nasser Alyemeni, Arif Shafi Wani, John Pichtel and Aquil Ahmad (2012). Role of proline under changing environments. *Plant Signal Behavior*, *7*(11), 1456-1466.

Sharanya, K. P., Ajith Kumar, K. G. and Parvathy, S. N. (2022). Recalcitrant behavior of the seeds of endangered *Syzygium zeyalanicum* (L.) DC. *Journal of Plant Growth Regulation*.

Smitha, G. R. and Das, M. (2016). Effect of seed moisture content, temperature and storage period on seed germination of *Saraca asoca*- An endangered medicinal plant. *Medicinal Plants*, *8*, 60–64.

Swain, T. and Hillis, W. E. (1959). The phenolic constituents of *Prunus domestica,* L. The quantitative analysis of phenolic constituents. *Journal Science Food and Agriculture*, *10*, 63 - 68.

Szabados, L. and Savouré, A. (2010). Proline: a multifunctional amino acid. *Trends in Plant Science*, *15*, 89 - 97.

Szuba, A., Kalemba, E. M., Wawrzyniak, M. K., Suszka, J. and Chmielarz, P. (2022). Deterioration in the Quality of Recalcitrant Quercus robur Seeds during Six Months of Storage at Subzero Temperatures: Ineffective Activation of Prosurvival Mechanisms and Evidence of Freezing Stress from an Untargeted Metabolomic Study. *Metabolites*, *12*(8), 756.

Trusiak, M., Plitta-Michalak, B. P. and Michalak, M. (2022). Choosing the Right Path for the Successful Storage of Seeds. *Plants*, *12*(1), 72.

Varghese, B. and Naithani, S. C. (2002). Desiccation-induced changes in lipid peroxidation, superoxide level and antioxidant enzyme activity in neem (*Azadirachta indica* A. Juss.) seeds. *Acta Physiologiae Plantarum, 24*, 79–87.

Varghese, B., Naithani, R., Dulloo, M. E. and Naithani, S. C. (2002). Seed storage behavior in *Madhuca indica* J. F. Gmel. *Seed Science and Technology*, *30*, 107-117.

Verbruggen, N. and Hermans, C. (2008). Proline accumulation in plants: a review. *Amino Acids*, *35* (4), 753 - 759.

Vertucci, C. W. and Farrant, J. M. (1995). Acquisition and loss of desiccation tolerance. In: J. Kigel and G. Galili (eds). Seed development and germination, Marcel Dekker Inc. New York, USA. 237-271.

Walters, C., Pammenter, N. W., Berjak, P. and Crane, J (2001). Desiccation damage, accelerated ageing and respiration in desiccation tolerant and sensitive seeds. *Seed Science Research*, *11,* 135–148.

Walters, C., Wheeler, L. and Stanwood, P. C. (2004). Longevity of cryogenically stored seeds. *Cryobiology*, *48*(3), 229–244.

Wen, B. (2009). Storage of recalcitrant seeds: a case study of the Chinese fan palm, *Livistona chinensis*. *Seed Science and Technology, 37,* 167-179.

Yadav, B., Jogawat, A., Rahman, M. S. and Narayan, O. P (2021). Secondary metabolites in the drought stress tolerance of crop plants: A review. *Gene Reports*, *23*, 101040.

How to cite this article: Suresh Kumar K.A., Anilkumar C., Ajithkumar K.G., Prajith T.M. and Amritha V.V. (2023). Extension of Seed Longevity of *Myristica malabarica* Lam. by Optimizing Storage Conditions. *Biological Forum – An International Journal, 15*(4): 155-161.