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Effect of desiccation damage on the seed viability of *Hydnocarpus alpina* Wight of Western Ghats

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Abstract

Hydnocarpus alpina Wight is an evergreen endangered medicinal tree species of Western Ghats. Several local names are available based on the distribution among the state in India that is Torathi (Kannada), Maravetti/Marotti (Malayalam), Attuchankalai (Tamil), Kastel (Hindi). *Hydnocarpus alpina* has very appreciated value in various medicinal properties like anti-larvicidal, anti-feedant, antimicrobial etc. due to the presence of significant chemical constituents. The seeds of this tree species are characterized with high moisture content and are intolerant to desiccation. They are under recalcitrant type seeds.

Fully mature *H. alpina* seeds have 20% moisture content on harvest and recorded 80% germination. After 2 weeks of desiccation at open laboratory conditions (28±2 °C and 65% RH) their moisture content and percentage of germination decreased to 10.2% and 20 % respectively. Complete loss of viability occurred at 10% moisture level. This clearly indicates the recalcitrant nature of seeds. Different biochemical parameters such as lipid peroxidation, leachate conductivity, assay of free radical scavenging enzymes, analysis of primary metabolites like total soluble sugars, starch, total proteins, amino acids, lipids, phenols etc. were recorded for finding the biochemical basis of desiccation induced damage on seed viability. The results of the study reveals the metabolic disturbances associated with the desiccation of seeds and such parameters can be considered as markers for assessing the seed viability. Hence, safest low moisture content for the long term storage of seeds can be identified for the conservation of this endangered species of Western Ghats.

Keywords

Desiccation, recalcitrant, tetrazolium, leachate, lipid peroxidation

Introduction

The Western Ghats are internationally recognized as a region of immense global importance for the conservation of biological diversity. A significant characteristic of the Western Ghats is the exceptionally high level of endemism. The forests in the Western Ghats are with rich diversity and the area is facing severe threat and endangerment due to various physical and physiological reasons. Of course, this also may be due to anthropogenic reasons. So, conservation of our plant wealth is utmost important for maintaining the sustainable environment. Some plants are found only to the Western Ghats. Identification of that particular plant species, its conservation and their restoration is equal to the conservation of the whole population.

Hydnocarpus alpina Wight is such an evergreen endangered medicinal tree species endemic to Western Ghats (29). Several local names are there based on the distribution among the state in India that is Torathi (Kannada), Maravetti/Marotti (Malayalam), Attuchankalai (Tamil), Kastel (Hindi). This tree has very appreciated value in various medicinal properties like anti-larvicidal, anti-feedant, antimicrobial etc. due to the presence of significant chemical constituents. The seeds of *Hydnocarpus alpina* are recalcitrant in nature and lose their viability within a few days after detachment from the mother plant. The tree grow-up to 8 m tall, the trunk and bark is dark grey, slightly rough; blaze light orange. Branches and branchlets subterete, glabrous, drooping. Leaves simple, alternate, distichous; petiole 0.7-1.6 cm long, swollen at both ends, canaliculate, glabrous; lamina 9-26 x 2.5-8.5 cm, usually lanceolate, apex gradually acuminate or subacute, base rounded or acute, sometimes sub attenuate, margin entire, coriaceous, glabrous; midrib raised above; secondary nerves 6-11 pairs, ascending towards apex; tertiary nerves reticulopercurrent. Flowers unisexual, solitary or in short fascicles and white in colour. Fruits and seeds are berry, globose, to 6.5 cm across, brown tomentose, pericarp woody; seeds numerous. Flowering and fruiting: February-July. The chemical constituents found in this species are chaulmoogric acid, hydnocarpic acid, apigenin, hydnocarpin, fixed oils, tannins etc. Its timber is good for construction purposes and is used for Beams and Rafters.

The seeds of *Hydnocarpus alpina* tree contain high level of fatty oil which is similar to chaulmoogra oil used extensively in the treatment of leprosy and other cutaneous diseases and also used as an illuminant. The Seeds are also characterized with high moisture content and are intolerant to desiccation. The loss of viability in recalcitrant seeds is the synergetic effect of a wide range of metabolic processes. These include mechanical stresses induced by the removal of water which can use structural changes at sub cellular, cellular and tissue levels. It was suggests that the activities of metabolites in the recalcitrant seeds are altered by drying, resulting in the loss of biological activity (1). Thus the quantification of this bio - molecules (TSS, Starch, Protein, Amino acids and Lipids) can be serve as the markers in ascertaining the seed viability.

Conservation of this recalcitrant tree species is very important due to its narrow endemism. *Hydnocarpus alpina* is such an endangered plant with recalcitrant type seeds. The seeds are mainly of two types ie. orthodox and recalcitrant seeds. Recalcitrant seeds shed at high water content, are desiccation sensitive and cannot be stored under conditions conventionally employed for desiccation -tolerant orthodox seeds. Recalcitrant seeds are metabolically active when shed, with high rates of respiration and a high degree of intracellular differentiation. Development grades gradually into germination, with no punctuating quiescent period and many recalcitrant seeds will germinate if maintained at their shedding water content (30).

Seed is the only propagation tool for this species, therefore the study of the seeds of this rare medicinal tree species is of utmost importance. Narrow endemism of the species also makes it to extinction; hence the need for conservation and restoration is relevant.

Materials and Methods

Desiccation studies

The mature fruits of *H. alpina* were collected from Kerala state, India. The fruits initiation starts during the months of January-February. But maturity attain after during rainy season (June- July). The fruit maturity and fruit drooping occurs. Seeds were collected from the ground after its natural shedding. The seeds are considered mature, when the seeds attained maximum dry weight (3), when they start shedding. Collected seeds were then de-pulped for further studies.

Moisture content analysis

Moisture content was determined by the Low Constant Air Oven Method (4). For dry weight determination, the seed material was taken in a pre - weighed bottle and weighed in an electronic balance, dried in a hot air oven at 103 ^{0}C for 17 hrs or more(until the constant weight were obtained) .

Leachate conductivity measurements

Electrolyte leakage was tested by taking three replicates of ten seeds for *H. alpina* under different desiccation periods (In days 1-20) The seeds were placed in glass beaker containing 25 ml distilled water and covered to reduce evaporation and contamination by dust and were kept in laboratory condition. After 24 hrs the seeds were removed, the electrical conductivity of the seed leachate was measured using a dip cell conductivity metre (Systronics, DDR type 306) specific conductance was expressed as µs cm $^{-1}$ g $^{-1}$.

Germination test were carried out in three replicates of 30 seeds in each rolled in acid free germination paper kept in seed germinator conditions 30 ± 2 ºC and 65 % RH.

Biochemical extraction and estimation

One gram fresh tissue sample from different desiccation period was homogenized in 80% ethanol. Homogenate was then centrifuged at 3000 rpm for 10 min. The residue was washed again with 80% ethanol. The volume of pooled supernatant was noted and served as the source for estimation of phenol (5) and amino acid (6). A known volume of combined ethanol fraction was evaporated to dryness and then the residue was re dissolved in known volume of distilled water by using a fine polished glass rod and served as the source for total soluble sugar estimation (7). The left over residue was ground with 30% and 15% Per Chloric Acid (PCA) respectively at 2 times, centrifuged at 3000 rpm for 10 min each and combined supernatant used for the estimation of starch (8). For protein estimation, the tissue was homogenized in double distilled water and precipitated with Tri Chloro Acetic acid (TCA) and estimated following (9).

Lipid extraction and estimation

Lipids were extracted following the method of Bligh and

Dyer (10). From the randomized sample of the seed, 1 gm dry tissue with a pinch of acid washed sterilized sand (as an abrasive) was ground to a fine paste using mortar and pestle with 10 ml of distilled water. The slurry was transferred to a conical flask (250 ml capacity) containing 30 ml of chloroform: methanol mixture (2:1 v/v) and mixed well. The flasks were incubated for overnight in dark at room temperature for complete extraction of lipids. After incubation the slurry was centrifuged at 5000 rpm for 15 min after adding 20 ml of distilled water. After centrifugation, of the 3 layers formed the clear lower layer of chloroform containing all the lipids was carefully collected. This was transferred to pre weighed china dish and carefully evaporated to dryness and determined the weight. The difference in weight gaves the weight of the lipid and expressed as mg total lipid $d.wt^{-1}$.

Tetrazolium reduction activity

A modification of method described by (11) was used. The slightly broken seeds, in triplicates of cotyledon were used. Seeds incubated in 1% 2, 3, 5 - triphenyltetrazolium chloride (in 10mM sodium phosphate buffer pH 7.2) in darkness for 4 hrs. After 3 washes in distilled water, 5 ml acetone was added and incubated at room temperature in darkness for 16 - 18 hrs to elute the formed formazan. The supernatant was filtered through whatman No 1 filter paper, and the colour was measured at 480 nm g.d.wt. Treated extracts from seeds incubated in absence of tetrazolium served as blank.

Lipid peroxidation

Lipid peroxidation was measured as the concentrations of thiobarbituric acid reactive substance (TBRS), equated with malon di aldehyde (MDA) (12). Fresh tissue (500 mg) was homogenated in 1 ml distilled water with sterilized silica in a mortar and pestle. Two ml of 5% thiobarbituric acid (TBA) (prepared in 20% trichloro acetic acid) was added to the homogenate. This reacting mixture was incubated in a boiling water bath for 30 min. A pink colour was developed by the reaction of various lipid peroxidation products (viz. MDA) with TBA. The reaction tubes were immediately introduced to a freezer (at 0 ^{0}C) for 15 min to stop the reaction. The colour of the supernatant after centrifugation (at 3000 rpm for 15 min) was measured at 540 nm and 600 nm respectively. After subtracting the absorbance of the nonspecific (at 540 nm) the net absorbance was expressed at 540 nm $g.f\ wt^{-1}$ of the tissue.

Extraction of enzymes

Fresh cotyledons (1 gm) from the seeds were 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 min and supernatant was precipitated in cold acetone (1:3 ratios) and kept in an ice bath for 30 min. The pellet was washed with acetone twice and re suspended in same buffer after complete evaporation of acetone. Centrifuged and the combined supernatant served as the enzyme source for both peroxidase and poly phenol oxidase. All enzyme extractions were carried out at 4 ^{0}C.

Peroxidase assay (H2O2 Oxidoreductase E.C.1.11.1.7)

Peroxidase activity was measured according to the method of (13), recording the change in absorbance at 470 nm due to oxidation of guaiacol in the presence of hydrogen peroxide. The assay mixture (3 ml) contained 0.1 M sodium phosphate buffer (pH 7.0), 0.02 M guaiacol and enzyme extract. The reaction was triggered by addition of 13 mM H_2O_2 to the mixture. The change in absorbance was recorded in a UV-VIS spectrophotometer (Pharmaspec UV-1700, Shimadzu, Japan) at 470 nm. The enzyme activity expressed as change in absorbance min^{-1} mg $protein^{-1}$.

Polyphenol oxidase assay (mono phenol, dihydroxy phenyl alanine: oxygen Oxidoreductase E C 1.14.18.1)

The method of (14) was followed to estimate poly phenol oxidase (O - di – phenol Oxidase) activity. The assay mixture consists of 0.01 M catechol and 0.1 M proline in Sodium phosphate buffer (pH 6.8). Just after the addition of enzyme extract (mixed quickly), the kinetics of the reaction was immediately recorded at 525 nm, by using UV-VIS spectrophotometer (Pharmaspec UV-1700, Shimadzu, Japan) and the enzyme activity was calculated as the initial linear change in absorbance min^{-1} mg $protein^{-1}$.

Catalase assay

a) Preparation of acetone powder

Seed tissues (1 g) were homogenated in a pre – chilled blender (mortar) and pestle with chilled acetone (-20 ^{0} C) enough to cover the tissue. The homogenate was filtered through a Buchner funnel using whatman No.1 filter paper. The paste was washed thrice with chilled acetone. The paste on Whatman No.1 filter paper was air dried for 1 hr. The powder is stored in containers with tight caps in a freezer. This powder can be used up to 6 months without loss of activity. The assay was done by grinding known amount of enzyme powder in 5- 10 ml of phosphate buffer (0.1 M and pH 6.8) in a mortar and pestle at 4 ^{0}C. The extract was centrifuged at 3000 rpm at 4 ^{0}C for appropriate time (5minutes), and the clear supernatant served as enzyme source. Protein content in the supernatant was estimated (9) and expresses the specific activity.

b) Assay of catalase enzyme

Catalase activity was measured according to the modified permanganometric method (15). The reaction mixture contained 3 ml phosphate buffer (0.1 M, pH 6.8), 1 ml hydrogen peroxide (1%) and 1 ml of enzyme extract. The reaction mixture was incubated for 1 min at 25 ^{0}C.

The reaction was terminated by adding 10 ml 2% H_2SO_4. This reaction mixture was titrated against 0.01N $KMnO_4$ to estimate the residual H_2O_2, until a faint purple colour persists for at least 15 seconds. Add enzyme extract to stop the reaction in control also. The enzyme activity is expressed in unit / g f.wt. under standard assay conditions.

Statistical analysis

The data from different experiments were analysed following one way Analysis of Variance (ANOVA) and the ratio obtained were checked for significance at 1 and 5 % probability (P) level. From this calculated ANOVA, the means of

each treatment were separated following the least significance Difference (LSD) by Duncan's multiple range test at 1 and 5% level.

Results

Fresh seeds of *H. alpina* on harvest had 20.1% moisture content. Seed moisture content and final germination percentage decreased considerably. Fresh seeds of the species showed 80% germination. After 16 days, the moisture content dipped to 10%, considered as Critical Moisture Content and the germination was reduced to 20% (Table 1).

Table 1. Desiccation response of *Hydnocarpus alpina* seeds

Desiccation periods (Days)	Moisture content (%) ± SE	Germination (%)±SE	Days required for germination (Germination energy)
1	20.1 ± 0.31***	80 ±0.39***	14
4	19.3 ±0.12***	75 ±0.33***	18
8	17.6 ± 0.12***	50 ±0.34***	25
12	13.6 ± 0.08***	40 ±0.34***	28
16	10 ± 0.033***	20 ±0.033***	45
20	3 ± 0.16***	NIL	……..

± SE: Standard Error of the Mean

Tetrazolium reduction activity (Formazan formation)

The decrease in moisture content according to the desiccation period showed difference in pattern and time required for staining with tetrazolium chloride (TTC) solution. Fresh seeds of *H. alpina* (MC 20.1 %) showed deep red colour on incubation in TTC for 30-45 min. With reduction in moisture content, the staining pattern showed variations and took more time for colour development (Fig. 1).

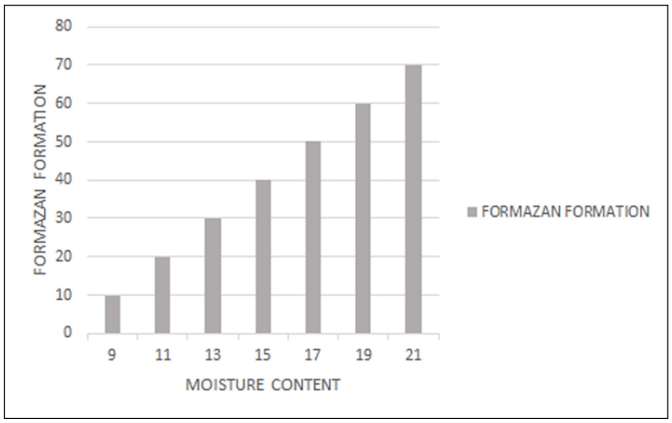

Fig. 1. Seed staining intensity with respect to moisture content of seeds of *H. alpina*.

Leachate conductivity

The electrical conductivity measurement showed an increased solute leachate from the seed tissues. The seed leachate collected from the desiccated *H. alpina* seeds at different intervals showed an increase in the leachate conductivity was correlated with the decreasing moisture content and germination. In control, the seeds of *H. alpina,* the specific conductance was only 12.6 µs, but after 384 hrs (i.e, 16 days) of desiccation, the leachate conductivity increased almost 6 fold (75.6 µs) (Fig. 2).

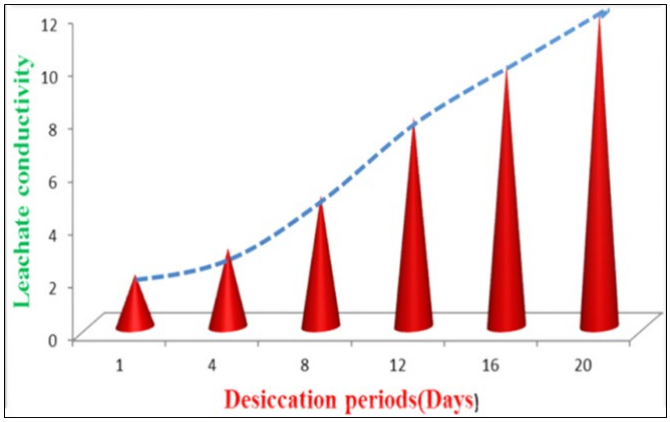

Fig. 2. Correlation of desiccation period with leachate conductivity in *H. alpina.*

Lipid peroxidation

The extents of lipid peroxidation in seeds of *H. alpina* were measured on the basis of production of Thiobarbituric acid Reactive Substances (TBRS) during different desiccation/ drying treatments. The production of TBRS is as a result of increased lipid peroxidation in the seeds of *H. alpina* during the entire desiccation period. This trend is similar to that of most of the other recalcitrant seeds (Fig. 3).

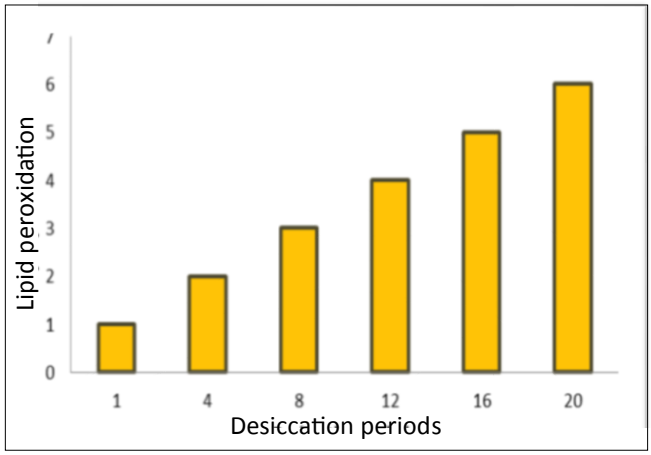

Fig. 3. Relation between the extents of lipid peroxidation in seeds of *H. alpina* with desiccation/drying treatments.

Quantitative estimation of biomolecules during desiccation

Total soluble sugar (TSS) content showed a gradual increasing trend, especially after 24 hrs of desiccation. But the starch content in the seeds of *H. alpina* was maximum at the initial stages of desiccation and then followed by a decreasing trend, finally the content become stabilized (Table 2). The total phenol content was minimum at the initial stages of desiccation, when the desiccation progressed the content of phenol gradually increased. Significantly high levels of protein were present at the initial stages of desiccation and then followed by a gradual decrease. Similarly, the amino acids were minimal at the initial stages of desiccation followed by a slight increase and finally it showed a decline and reaches nearly the initial level. The amount of lipid is slightly increases when the desiccation progresses. Thereafter to a particular period the content of lipid decreases and finally it get stabilized (Table 2).

Table 2. Quantitative estimation of bio molecules during desiccation of *Hydnocarpus alpina* seeds

Desiccation periods (Days)	Total soluble sugar mg g^{-1} d.wt ± SE	Starch mg g-1 d.wt ± SE	Total phenol mg g-1 d.wt ± SE	Protein mg g-1 d.wt ± SE	Aminoacid mg g-1 d.wt ± SE	Lipid mg g-1 d.wt ± SE
1	2.971 ±0.05*	77.66±10.20*	4.75 ± 0.05*	37.8 ± 0.51*	5.2 ± 0.1**	304 ± 0.125
4	5.3 ± 0.20*	76.12±10.05*	8.64 ± 0.80*	35.66 ±8.41*	8.2 ± 0.20*	306 ± 1.20
8	10.2 ± 0.25*	74.32 ± 9.10*	9.25 ± 0.21*	24.4 ± 0.26*	8.7 ± 1.01	301 ± 1.00
12	14.32 ±0.22*	69.01 ± 8.17	10.21±0.25*	24.3 ± 0.29*	8.6 ± 0.21*	294 ± 0.221
16	18.58 ±0.31*	62.21 ± 8.01	10. 01±0.10*	21.8± 1.06	6.9 ± 0.10*	290 ± 0.220
20	20 ± 0. 50*	61.02±7.09**	12.46 ±0.21*	21.7 ± 0.30*	5.8 ± 0.30*	284 ± 0.22

± SE: Standard Error of the Mean, Significance level *

Enzyme activity during desiccation

The antioxidant enzymes showed higher activity during the initial period of desiccation. The activities of all the three enzymes doubled during desiccation (8-16) days. Subsequent desiccation results a sharp decrease of all of the enzymes. Then, it keeps a static value. Comparatively the activity of catalase is high during desiccation (Table 3).

Table 3. ROS – Anti oxidant enzyme assay during desiccation of *Hydnocarpus alpina* seeds

Desiccation periods (Days)	Peroxidase activity (A470/ min./mg protein)	Poly phenol oxidase (A525/ min./mg protein)	Catalase (Unit per gm.fwt)
1	0.088 ± 0.07**	0.007 ± 0.002***	0.003 ± 0.001***
4	0.10 ± 0.02*	0.087 ± 0.027**	0.138 ± 0.012***
8	0.364 ± 0.04*	0.112 ± 0.16*	0.933 ± 0.13*
12	0.302 ± 0.02*	0.120 ± 0.02**	0.871 ± 0.12*
16	0.091 ± 0.08**	0.031 ± 0.001***	0.051 ± 0.10*
20	0.086 ± 0.06**	0.008 ± 0.004***	0.004 ± 0.002***

± SE: Standard Error of the Mean

Discussion

Seed desiccation studies in *Hydnocarpus alpina* gauged by changes in fresh weight and moisture content levels pertaining to viability followed a pattern of typical recalcitrant nature. Desiccation sensitive or 'recalcitrant' seeds do not tolerate drying and are hardly storable (16). Consequently, the use and conservation of recalcitrant-seeded species, which include some economically important plants, remain a challenge (17). In *H. alpina*, the moisture content reduced when the seeds are desiccated. After 16 days of desiccation, the moisture content reduced to 10 % with 20% germination. Further, seed desiccation does not show any germination. The decrease in germination percentage is due to desiccation stress. In *H. alpina*, there is a direct correlation found between moisture content and germination percentage. ie, when the moisture content is high then the % of germination was also high. This results are in line with the findings of (2, 18) in *H. pentandra.*

In *H. alpina* seeds, the prolonged desiccation shows an increase in solute leakage. It may be due to loss of membrane integrity. The cell membrane integrity is considered one of the primary physiological events of seed deterioration process (19). Maximum specific conductance of *H. alpina* seeds were 75.6 µs was recorded during the 16th day of desiccation.) Low conductivity means a high-quality seed and high conductivity, that is greater seed leachate, suggests that less force (19). The tetrazolium (TTC) test is also known as a biochemical test used to estimate the viability and seed germination. In *H. alpina* seeds at high moisture content, stains rapidly compared to desiccated ones. The desiccation also affects the staining pattern and the formazan formation also indicated the different desiccation periods and the loss of viability of the seeds.

Increase of TSS during the course of seed desiccation in *H. alpina* seeds is a feature related to the desiccation sensitive seeds, which in agreement with the findings in the seeds of cocoa, jackfruit and tea, which showed a decline in viability and moisture level associated with increased leachate conductivity and soluble carbohydrate (20). The present study revealed that the significant decrease in starch during desiccation is in line with the observations (21). After harvest, the seeds of *H. alpina* are metabolically active with high levels of carbohydrates especially starch and sugars. Similarly protein and lipid content were also recorded. Estimation of protein in *H. alpina* seeds revealed significant reduction in total proteins. Reduced level of total protein content during desiccation in *H. alpina* seeds is in conformity with the results (22) in *Hopea parviflora* and *Vateria indica* (23, 24). The decrease of protein levels recorded was coincided with an increase of amino acids during the initial period of drying. It was suggested that the amino acid formation in the initial period of drying, due to the degradation of proteins proceeded towards the nonviable nature of the seeds (21). The lipid content in *H. alpina* seeds is very high during the initial period of desiccation and the widespread occurrence of this increase and its role closely associated with its membrane integrity. The present observation on the level of lipids decreased with desiccation draws parallel with the study in *H. pentandra* (18).

The antioxidant system plays a key role in scavenging excess reactive oxygen species (ROS) during seed desiccation (25). Under normal water content, the biological production and elimination of free radicals maintain dynamic equilibrium, but when seeds are subjected to water stress, the electron transfer of cells is blocked, so stress can generate and cause excessive accumulation of reactive oxygen species (O_2, H_2O_2, OH and O_2) (26). Superoxide dismutase (SOD), peroxidase (POD), Poly phenol oxidase and

other antioxidant enzymes play an extremely important role in scavenging excess ROS (27). In *H. alpina* seeds, the activities of POD, PPO were enhanced during the initial period of drying up to 8th day of desiccation. The catalase activity shows similar pattern. The activity of catalase was comparatively higher than other 2 enzymes during desiccation. As these enzymes basically function as free radical scavenging enzymes, during initial period of drying there may be an increased level of free radicals and increased activities of POD, PPO and CAT for scavenging the free radical thereby keeping the seed viable up to a certain level. Similar enzymatic changes were found in *H. pentandra* and in *Ginkgo biloba* (18, 28).

Conclusion

The physiological as well as biochemical studies provide firm evidence that the seeds of *H. alpina* exhibit recalcitrant seed viability characteristics. The present study revealed that the seed desiccation induce drastic metabolic variations, especially the level of total soluble sugar, protein and lipid contents. Increased lipid peroxidation rate, activities of free radical scavenging enzymes, electrolyte leakage etc. might occur with the desiccation of seeds. Thus active metabolism and related changes occur during desiccation of seeds may be considered as the markers for detecting seed viability. Germination studies were carried out after desiccation, which reveals that the acid free germination paper and riverine soil gaves the maximum growth of the tree species.

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Authors contributions

CRV conducted the study and prepared draft manuscript and under the supervision of RM who finalized the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None.

References

1. King MW, Roberts EH. The storage of recalcitrant seeds - achievements and possibleapproaches. International Board for Plant Genetic Resources. 1979;p-96.
2. Manasi RN, Channabasappa K. Effect of storage period on seed germination of *Hydnocarpus pentandra* (Buch-Ham). Journal of Agricultural Science.2014;26(2):1-2.
3. Li C, Sun WQ. Desiccation sensitivity and activities of free radical scavenging enzymes in recalcitrant *Theobroma cacao* seeds. Seed Science Research.1999;9:209-17. https://doi.org/10.1017/S0960258599000215
4. ISTA. International Rules for Seed Testing. Seed Science and Technology. 1985;13(2):338-41.
5. Swain T, Hillis WE. The phenolic constituents of *Prunus domestica* I.-The quantitative analysis of phenolic constituents. Journal Science Food and Agriculture.1959;10:63- 68. https://doi.org/10.1002/jsfa.2740100110
6. Lee, Takahashi. An improved colorimetric determination of amino acids with the use of Ninhydrin, Analytical Biochemistry.1966;14(1):71-77. https://doi.org/10.1016/0003-2697(66)90057-1
7. Montgomery R. Determination of glycogen. Archives of Biochemistry and Biophysics. 1957;67:378-86. https://doi.org/10.1016/0003-9861(57)90292-8
8. McCready RM, Guggolz J, Siliera V, Owens HS. Determination of starch and amylase in vegetables. Analytical Chemistry. 1950;22:1156-58. https://doi.org/10.1021/ac60045a016
9. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin – Phenol reagent. Journal of Biological Chemistry. 1951;193:265-75. https://doi.org/10.1016/S0021-9258(19)52451-6
10. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology. 1959;3:911-17.https://doi.org/10.1139/o59-099
11. Kittock DL, Law AG. Relationship of seedling vigour, respiration and tetrazolium chloride reduction by germinating wheat seeds. Agronomy Journal. 1968;60:286-88. https://doi.org/10.2134/agronj1968.00021962006000030012x
12. Heath RL, Packer L. Photo - peroxidation in isolated chloroplasts 1. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics. 1968;125:189-98. https://doi.org/10.1016/0003-9861(68)90654-1
13. Chance B, Maehly AC. Assay of catalases and peroxidases. Methods in Enzymology. 1955;2:764-75. https://doi.org/10.1016/S0076-6879(55)02300-8
14. Yamaguchi M, Hwang PM, Cambell JD. Latent O-diphenol oxidase in mushrooms (*Agaricus bisporus*). Canadian Journal of Biochemistry. 1970;48:198-202.https://doi.org/10.1139/o70-032
15. Kar M, Mishra D. Catalase, peroxidase and polyphenol oxidase activities during rice leaf senescence. Plant Physiology. 1976;5:315-19. https://doi.org/10.1104/pp.57.2.315
16. Roberts EH. Predicting the storage life of seeds. Seeds Science and Technology. 1973;1:499-514.
17. Berjak P, Pammenter NW. From Avicennia to Zizania: seed recalcitrance in perspective. Annals of Botany. 2013;101(2):213-28. https://doi.org/10.1093/aob/mcm168
18. Kamarudeen M, Jee G. Influence of desiccation on germination and biochemistry in seeds of *Hydnocarpus pentandra* (Buch-Ham.) Oken, an endemic and endangered medicinal tree species of Southern Western Ghats. Abrahamia. 2016;2:73-79.
19. Vieira RD, Krzyzanowski FC, FrançaNeto JB. Electrical conductivity test, in Seed Vigor: Concepts and Tests, Eds. 1999;26(4):1-4.
20. Chandel KPS, Rekha Chaudhury, Radhamani J, Mlik SK. Desiccation and freezing sensitivity in recalcitrant seeds of Tea, Cocoa and Jackfruit. Annals of Botany. 1995;76:443-50. https://doi.org/10.1006/anbo.1995.1118
21. Bewley JD, Black M. Physiology and biochemistry of seeds 2 -

Viability, Dormancy and Environmental control, Springer - Verlag. Berlin. 1982;Vol. 2.

22. Kamarudeen M. Physiological and biochemical studies on the seeds of *Hopea parviflora* Bedd. and *Vateria indica* L. two economically important endemic trees of Western Ghats. PhD thesis work, University of Kerala. 2003.

23. Finch - savage WE, Blake PS, Clay HA. Desiccation stress in recalcitrant *Quercus robur* L. Seeds results in lipid peroxidation and increased synthesis Jasmonates and Abscisic acid. Journal of Experimental Botany. 1996;47:661-67. https://doi.org/10.1093/jxb/47.5.661

24. Chaitanya KSK , Keshavkant S, Naithani SC. Changes in total protein and protease activity in dehydrating recalcitrant sal (*Shorea robusta*) seeds. *Silva fennica*. 2000;34(1):71- 77. https://doi.org/10.14214/sf.646

25. Bailly C, Boghatek-Leszczynska R, Come D, Corbineau F. Changes in activities of antioxidant enzymes and lipoxygenase during growth of sunflower seedlings from seeds of different vigour. Seeds Science and Research. 2002;12(1):47-55. https://doi.org/10.1079/SSR200197

26. Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology Biochem. 2010;48:909-30. https://doi.org/10.1016/j.plaphy.2010.08.016

27. Sharma P, Jha AB, Dubey RS, Pessarakli M. Reactive oxygen species, oxidative damage and antioxidative defense mechanism in plants under stressful conditions. Journal of Botany. 2012;p.21-37. https://doi.org/10.1155/2012/217037

28. JingFeng, YongbaoShen, Fenghou Shi, Chengzhong Li. Changes in seed germination ability, lipid peroxidation and antioxidant enzyme activities of *Ginkgo biloba* seed during desiccation. Forest. 2017;286(8):1-13. https://doi.org/10.3390/f8080286

29. Karuppuswamy S. Diversity and conservation status of red listed medicinal plants in Tamilnadu, Kongunad Research Journal. 2018;5(2):41-49. https://doi.org/10.26524/krj269

30. Norman WP, Patricia Berjak. Development of the understanding of seed recalcitrant and implications for *ex situ* conservation Biotecnología Vegetal. 2013;13(3):131-44.

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