Syncing phenology phase and canopy spectral reflectance of common tree species of four forest covers in India

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Variability in the leaf phenology of tropical trees impacts their growth. How phenology of tree species responds over rainfall gradient is relevant to study in the light of current climatic changes. Airborne visible and infrared imaging spectrometer-next generation (AVIRIS-NG) spectral datasets have been considered for this study as they not only provide wider area of coverage, but also high spatial and spectrally resolved output. Canopy-level spectra of 16 common species of four different forest covers in India were synced with observed phenology phase and the annual rainfall in each forest cover was recorded. Reflectance spectra of the same species in the four forest covers distinctively differed over rainfall gradient, indicating intra-species pliability. Consistent lower reflectance/higher absorption at chlorophyll bands of all the common deciduous species in the higher annual rainfall region over that with relatively lower rainfall indicated that deciduous species acclimate green foliage phase of the phenology cycle. Boxplots of reflectance values of chlorophyll absorption band of 16 species showed a decrease in the variability of the datasets over the four forest covers, revealing that increasing rainfall provides better synchrony in the phenology phase of the observed tree species. The study highlights the importance of AVIRIS-NG spectral datasets in monitoring different phases of forest phenology associated with growth potential dynamics effectively under changing rainfall pattern.

Keywords: Absorption band, canopy-level spectra, forest cover, phenology phase, rainfall gradient, tree species.

PHENOLOGY of trees indicates regular annual events starting from bud break until leaf fall. It is a major temporal event in the growth cycle of trees and has a bearing on ecosystem functioning[1]. Tropical countries like India have a wide range of annual rainfall mixed with dry periods stretching up to 9 months a year. Naturally occurring tree species in India grow in dry deciduous to moist deciduous to evergreen forests with varied rainfall and dry season length. It is important to understand how common species modulate their phenological cycle across annual rainfall range of 900–2500 mm, and what bearing it has on the local community structure, growth and sustenance of forest covers. Observing land surface phenology is vital to perceive ecosystem response to the climate[2]. This can give valuable information about how species respond to environmental variabilities, specifically to rainfall, currently considered as a major climate change event in the tropics.

Remote sensing has immense potential in 'wall to wall' coverage of forest covers[3]. The recently carried out NASA (National Aeronautics and Space Administration) – ISRO (Indian Space Research Organisation) joint AVIRIS-NG (Airborne visible and infrared imaging spectrometer-next generation) campaign has provided high spectral and spatial resolution data of Indian forest covers[4] (https://vedas.sac.gov.in/aviris_2.0/sitemap.html). Spectroscopy can provide swift and effective ways to estimate canopy characteristics such as pigmentation, moisture content and cell-wall constituents from their optical attributes. Hyperspectral reflectance spectra of plants give better information about their biophysical and biochemical features, and how they vary because of biotic and abiotic factors[5]. Reflectance spectra from top of the canopy are impacted by canopy shape and thickness[6,7]. Canopy reflectance in the visible region indicates photosynthesis activity and in the near-infrared region (NIR) it is mostly influenced by structural properties of the canopy[5]. Assessing these features in the spectra of tropical trees indicates the response of their growth cycle towards rainfall variability.

There is limited information about diversity in tree phenology of different species growing in diverse regions, more specifically in the tropical forests[1]. An important aspect to study is how different features happening at canopy scale can be linked to the reflectance spectra[5]. Availability of high-resolution AVIRIS-NG data for four forest covers with varied annual rainfall and dry season length made this study possible. We studied the synchrony between the observed phase of phenology of tropical trees and measured canopy spectral reflectance over a rainfall gradient. The objective of this study was to test whether the phenology phase of common tree species in the four forest covers is similar over the rainfall gradient recorded, and how it gets captured in high-resolution canopy scale reflectance spectra of AVIRIS-NG datasets obtained within a narrow time window.

This study has been carried out in four forest covers of India, viz. Shoolpaneshwar Wildlife Sanctuary (SWS) and Vansda National Park (VNP) in Gujarat, Mudumalai Tiger Reserve (MTR) in Tamil Nadu, and Sholayar Reserve Forest (SRF) in Kerala. Annual rainfall and dry season length at the time of AVIRIS-NG data acquisition

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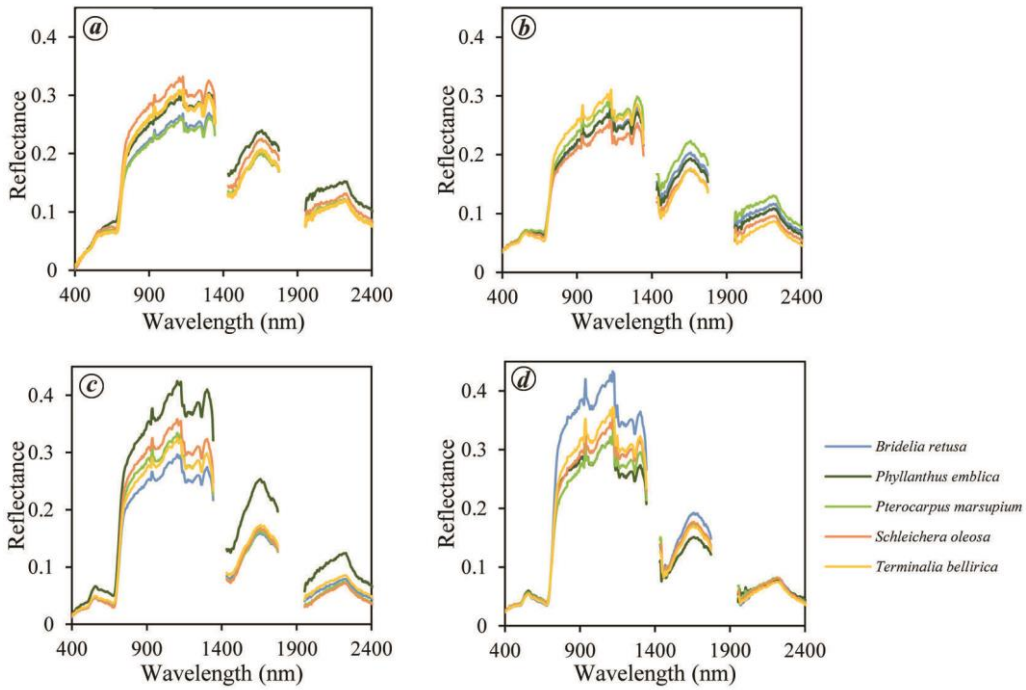

Figure 1. Mean canopy spectra (n = 2–50) of 5 of the 16 common species showing inter-species spectral variability: ***a***, Shoolpaneshwar Wildlife Sanctuary (SWS); ***b***, Vansda National Park (VNP); ***c***, Mudumalai Tiger Reserve (MTR); ***d***, Sholayar Reserve Forest (SRF).

Table 1. List of 16 common tree species across the forest covers: Shoolpaneshwar Wildlife Sanctuary, Vansda National Park, Mudumalai Tiger Reserve and Sholayar Reserve Forest

Botanical name	Family
Albizia odoratissima (L. f.) Benth.	Fabaceae
Albizia saman (Jacq.) Merr.	Fabaceae
Bamboo species	Poaceae
Bombax ceiba L.	Malvaceae
Bridelia retusa (L.) A. Juss.	Phyllanthaceae
Cassia fistula L.	Fabaceae
Dalbergia latifolia Roxb.	Fabaceae
Gmelina arborea Roxb.	Lamiaceae
Grewia tiliifolia Vahl	Malvaceae
Lagerstroemia lanceolata Wall.	Lythraceae
Mangifera indica L.	Anacardiaceae
Phyllanthus emblica L.	Phyllanthaceae
Pterocarpus marsupium Roxb.	Fabaceae
Schleichera oleosa (Lour.) Oken.	Sapindaceae
Tectona grandis L. f.	Lamiaceae
Terminalia bellirica (Gaertn.) Roxb.	Combretaceae

in the four forest covers were 978.75 mm, 270 d at SWS, 1107.91 mm, 240 d at VNP, 1486.28 mm, 120 d at MTR, and 2439.72 mm, 90 d at SRF respectively. Rainfall data were obtained from CHIRPS (Climate Hazards Group InfraRed Precipitation with Station data) (at 0.05° grid scale)[8]. The four forest covers are represented by dry deciduous to moist deciduous to evergreen forests. Most of the tree species spread over these four forest covers are deciduous, with some evergreen species. Proportion of evergreen tree species is relatively higher at MTR and SRF compared to the other two forest covers. Field studies were carried out in the four forest covers matching with the time window of AVIRIS-NG data acquisition (date of image data acquisition: MTR – 5/01/2016, SRF – 6/01/2016 and SWS – 8/02/2016 and VNP – 9/02/2016). Subsequent field visits were carried out towards incremental addition of plot-level data. Tree species diversity recorded in the field study over the four forest covers ranged between 68 and 80. Among these, 16 species were common in all four forest covers with varied distribution (Table 1). Field data (tree density, canopy spread and basal area) showed that occupancy of these 16 common species over the four forest covers ranged between 25% and 45%. Canopy spread of these 16 species across the four forest covers ranged between 4.02 and 638.20 m^2. The generated field data were utilized to classify AVIRIS-NG datasets. Ground-truth points of plots and of tree species were collected using Garmin Montana 650 GPS with ±3 m accuracy. Prior to classification, AVIRIS-NG datasets were pre-processed at the University of Wisconsin-Madison, USA (code is available at https://github.com/EnSpec/HyTools-sandbox). Processing of image data was carried out using ENVI image processing system (ENVI 5.3, Exelis Visual Information Solutions, Inc., USA). Support vector machine model classification was followed[9], and the accuracy of classification of tree species in the four forest covers was >78%. Canopy spectra (2–50 for each of the 16 common species at each forest cover) were obtained both from

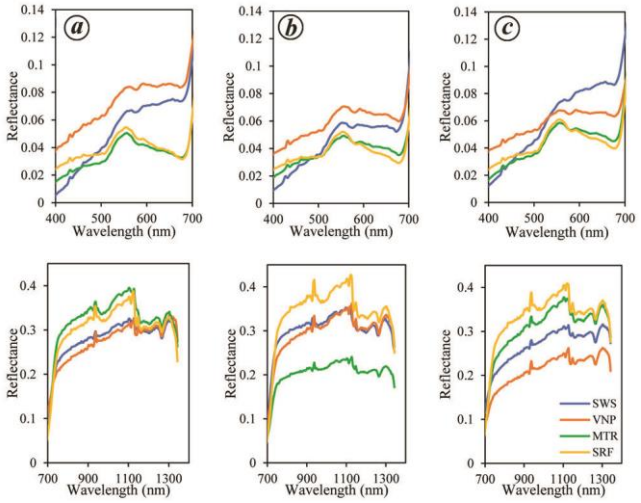

Figure 2. Intra-species spectral variability over the four forest covers in the spectral region 400–700 and 700–1400 nm: ***a***, *Albizia odoratissima*; ***b***, *Albizia saman*; ***c***, *Tectona grandis*.

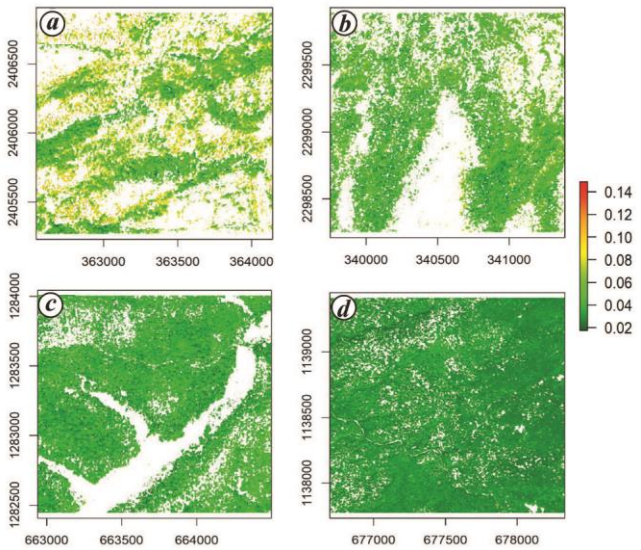

Figure 3. Map of reflectance measured at chlorophyll absorption band (676.96 nm) showing phenology phase variations in the four forest covers: ***a***, SWS; ***b***, VNP; ***c***, MTR; ***d***, SRF.

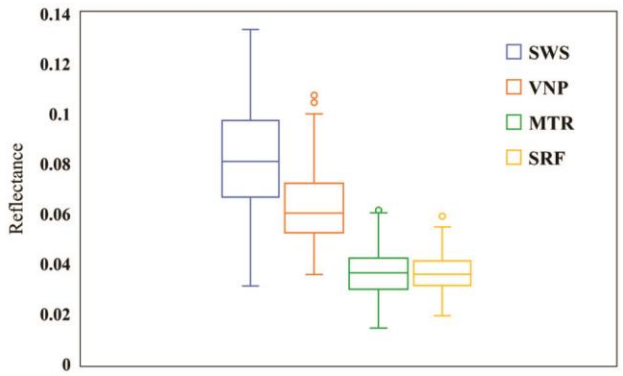

Figure 4. Boxplots (n = 200, from spectra of 16 common species) developed from reflectance values at 676.96 nm.

post-classification image and from ground-truth points collected during the field study. Mean spectra were obtained to address the objective of the study.

Deciduous characteristics of trees and their impact on phenology phase differed in the four forest covers because of variability in dry season length and annual rainfall recorded. Figure 1 shows the mean spectra of species of each forest cover and phenology phase variability across the four forest covers is given in the Supplementary Figure 1. Variability in the spectral reflectance between 700 and 1400 nm wavelength was largely because of inter-species canopy structure dynamics. Spread of the canopy spectra in the chlorophyll absorption band (676.96 nm) was much higher in SWS and it gradually tapered from VNP to MTR to SRF. Higher canopy reflectance in the chlorophyll absorption band indicated that canopy phenology was in the senescent phase. Rainfall is largely responsible for modulating both inter-species, and inter-cover phenology phase and observed canopy spectral features depict the influence of rainfall in modulating between species, and between forest cover phenology phase. Differences in the response of 16 common species seen in the four forest covers indicate forest-cover specific, rainfall-dependent investment strategy of species in regulating their phenology[1]. Intra-specific variation was distinctive in the reflectance curves over the four forest covers (Figure 2), indicating complexity in the species phenological responses. Species of the same genus also showed distinctive patterns (Figure 2).

Figures 3 and 4 show fluctuations in the reflectance values recovered at the chlorophyll absorption band (676.96 nm). The number of pixels with minimum reflectance in the chlorophyll absorption band was maximum for SRF (>70% of pixels) showing effective modulation of phenology phase of common deciduous species over rainfall gradient. Boxplots developed from reflectance values at 676.96 nm for the 16 species (200 canopy spectra for each cover) showed higher median value with greater spread in SWS, and lower median value with smaller spread in SRF. Results of this study show how phenology phase variability seen on the ground can be perfectly captured by high-resolution AVIRIS-NG data. Deciduous species growing in the four forest covers alter their phenology phase in sync with rainfall dynamics. This feature makes them more adaptive, broadly indicating the functional diversity of these forest covers.

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Effective use of synthetic seed technology in the regeneration of *Cymbidium aloifolium* using protocorm-like bodies

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Synthetic seed technology offers tremendous potential in micropropagation. It deals with *in vitro* conservation and storage of rare and endangered plant species along with their easy handling and transportation. This technology is becoming prevelant due to its wide applications in germplasm conservation and for exchanges between countries in floricultural trade. The present study examines the regeneration and conversion capabilities of *Cymbidium aloifolium* using protocorm-like bodies when stored at different temperatures. The propagules showed high proliferative potential by multiplication and complete plantlets were obtained in 58 days on basal M medium supplemented with 1 mg l^{-1} of indole-3-acetic acid.

Keywords: *Cymbidium aloifolium*, protocorm-like bodies, regeneration, synthetic seeds.

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THE development of an artificial seed technique provides a good approach for enhancement of various plant species such as trees and crops[1]. It represents a unique system for exploiting the inherent polyembryonate potential of orchids as well[2,3]. This method is advantageous as it combines clonal propagation and seed propagation with the possibility of long-term storage of seeds through encapsulation in a gel-like matrix[4]. In this technique, non-embryogenic vegetative propagules such as shoot tips, nodal segments or axillary buds, protocorm-like bodies (PLBs) or calluses are artificially encapsulated using sodium alginate as the preferred coating agent. According to Sharma *et al.*[5], these synthetic hydrated seeds contain nutrients that will help in the survival and speedy growth of embryos into plantlets during their cultivation after storage. This cost-effective method has proven to be quite productive, specifically for a number of orchid species[6–14]. The efficacy of synthetic seeds was successfully tested in *Cymbidium aloifolium* using PLBs, as they are easy to store and have the ability to divide as well as the best regeneration capacity which makes them ideal explants for regeneration and conservation in orchids. *C. aloifolium* is an Indo-Malayan, aloe-leaved, elegant epiphyte which has long earned the attention of herbalists for its therapeutic importance. According to Lawler[15], it is incorporated as one of the components of an oil formulation to treat tumours which are both benign and malignant. It is also used to cure eye ailments, vertigo and paralysis. The genus figures among the endangered orchids, enlisted in Appendix II of CITES[16], due to continuous destruction of its natural habitats, overexploitation for medicinal purposes, unauthorized trade and collection by orchid-lovers. The present study is a step forward to save the germplasm of this species using synthetic seed technology. The objective of the study is to determine the effects of different growth additives and different storage times in the regeneration of *C. aloifolium*.

The shoot-tip derived PLBs (measuring 0.2–0.3 cm in length) procured from *in vitro*-raised cultures were used to prepare synthetic seeds. Hence, these propagules did not require prior sterilization. The physical characteristics of the beads were controlled by the concentration of sodium alginate and calcium chloride used to form the calcium alginate gel (sodium alginate: 2–5% and Calcium chloride: 50–100 mM). The propagules were dispersed in the sodium alginate solution. The suspension was then added dropwise (each drop having a propagule) using a wide-mouthed pipette (10 ml) to the magnetically stirred calcium chloride solution. The beads were complexed for 30 min with periodic swirling. The resultant synthetic seeds were thoroughly washed with sterilized distilled water and initially cultured on basal (M; Mitra *et al.*[17]) medium for four weeks and subjected to 30 min mild dehydration prior to encapsulation.

The conversion frequency of these seeds was tested using only M medium after definite time periods (15 days

Table 1. Effect of different growth additives on time taken for initiation response and plantlet formation (days) in synthetic seeds immediately after their preparation in *Cymbidium aloifolium*

Additives (1 mg l^{-1})	Time taken for initiation response (days)	Time taken for plantlet formation (days)	Remarks
M	25	45	Protocorm-like bodies (PLBs) multiplication
M + IAA	38	58	Formation of plantlets with long roots
M + 2,4-D	34	54	–
M + BAP	30	51	PLBs multiplication
M + IAA + KN	40	60	PLBs multiplication
M + IBA + BAP	42	62	PLBs multiplication

M, Mitra medium; IAA, Indole-3-acetic acid; 2,4-D, 2,4-Dichlorophenoxyacetic acid; BAP, 6-Benzylaminopurine; IBA, Indole-3-butyric acid; KN, Kinetin.

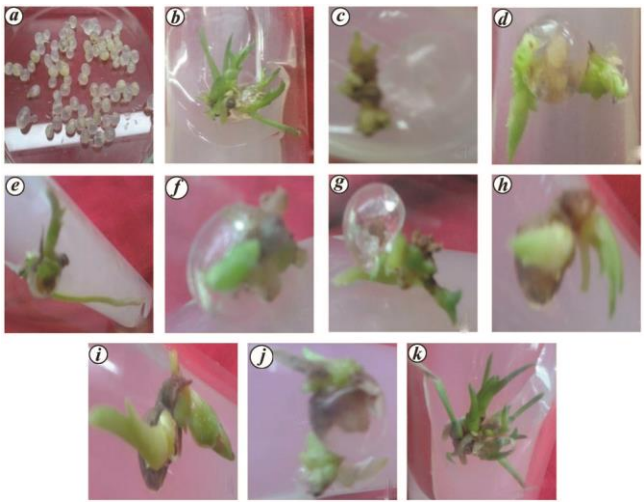

Figure 1. Synthetic seeds in *Cymbidium aloifolium*: ***a***, Spherical, non-leaky and firm seeds with 3% sodium alginate and 100 mM calcium chloride. ***b***, Multiple shoot formation (M + BAP (1 mg l^{-1})). ***c***, Formation of protocorm-like bodies (PLBs) (M + IBA (1 mg l^{-1}) + BAP (1 mg l^{-1})). ***d***, ***e***, Formation of long roots and complete plantlet formation (M + IAA (1 mg l^{-1})). ***f***, ***g***, Multiplication of PLBs (M + IAA (1 mg l^{-1}) + KN (1 mg l^{-1})). ***h***, ***i***, Formation of leaf primordia (M + 2, 4-D (1 mg l^{-1})). ***j***, Multiplication of PLBs and complete plantlet formation (M). ***k***, Complete plantlet formation with well-developed roots (M + BAP (1 mg l^{-1})).

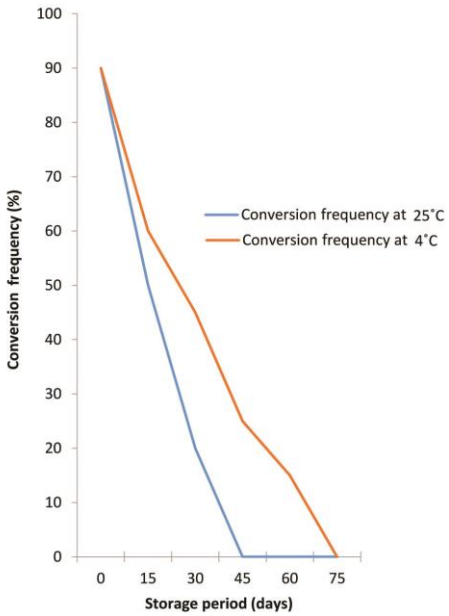

Figure 2. Effect of temperature and storage on the conversion frequency of synthetic seeds in *Cymbidium aloifolium.*

interval) to ascertain the maximum period for which the seeds could remain viable. The per cent viability of seeds was calculated by dividing the live seed count by total seed count. The seeds were stored at two different temperature regimes, viz. 4°C and 25°C.

Freshly prepared seeds were inoculated on basal M medium with and without different plant growth regulators (PGRs) into 20 × 150 mm culture tubes which were maintained at 25 ± 2°C under 35 μ E m^2 s^{-1} light intensity and 50–60% relative humidity. One set of encapsulated PLBs was kept in a refrigerator at 4°C and another set were kept at 25°C. Each treatment consisted of eight replicates and observations were made by taking the average time of all replicates. The experiment was repeated twice.

In the present experiment, synthetic seeds were successfully prepared in *C. aloifolium*. Their physical characteristics such as size, shape and firmness varied with the concentration of the gelling agent and quantity of calcium chloride used. An encapsulation matrix of 3% sodium alginate and 100 mM calcium chloride yielded spherical, non-leaky and firm seeds. Lower concentrations (sodium alginate; 2.0%, 2.5% and $CaCl_2$; 50 mM) were not suitable for encapsulation as the beads formed were irregularly outlined, soft and leaky. The effect of different PGRs on the time taken for initiation response and subsequent plantlet development (days) in synthetic seeds, immediately after their preparation was analysed. Table 1 and Figure 1 *a–k* provide a summary of the results. Freshly encapsulated PLBs (i.e. control) converted with 90% frequency after 25 days, when directly inoculated on M medium supplemented with different growth additives. The encapsulants, i.e. PLBs multiplied and differentiated into complete plantlets in 45 days. The propagules showed high proliferative potential by their multiplication and complete plantlets were observed in 58 days on basal medium supplemented with indole-3-acetic

acid (IAA) (1 mg l^{-1}). However, a combination of indole-3-butyric acid (IBA) and kinetin (KN) at 1 mg l^{-1} each resulted in delaying the initiation response and subsequent plantlet formation, which is in accordance with the results of Vij and Aggarwal[18] in *Vanda coerulea*. According to Vij[2], the exogenous requirement of plant hormones depends on their endogenous level in the plant system, which varies with the phase of plant growth.

The conversion frequency of seeds was also observed to vary with time and temperature of storage (Figure 2). The freshly prepared seeds converted readily on M medium and showed proliferation. Synthetic seeds stored at 4°C maintained their viability for a longer time compared to those stored at 25°C in *C. aloifolium*. Synthetic seeds retained 60% viability after 15 days, which gradually reduced to 45% after 30 days, 25% after 45 days and only 15% seeds converted after 60 days. However, seeds when stored at 25°C completely lost their viability after 45 days. Similar results were observed by Sarmah *et al.*[19] and Pehwal *et al.*[10] for seeds stored at 4°C. This is possibly due to low metabolic rates at low temperatures in accordance with an earlier suggestion[3]. According to Sakamoto *et al.*[20], synthetic seeds dry quickly and are difficult to store for longer periods unless kept in humid environment and/or coated with a hydrophobic membrane, coating of substances like wax, resin, polyorganosilicane, etc. has been used by some workers[21–23]. However, we did not perform such experiments due to paucity of time.

Hence, if we want to store synthetic seeds for longer period, coating of substances like wax, resin, etc. should be used and then stored in refrigerators for their longer viability.

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