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Moisture sorption isotherms and comparative storage longevity of Corchorus olitorius and C. capsularis accessions under genebank conditions

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Abstract

Ex situ conservation of jute mallow genetic resources depends on a reliable estimate of how long stored seeds remains viable. Comparative longevity assays based on controlled deterioration conditions only deliver meaningful contrasts when every sample enters storage at the same moisture content. This study constructed moisture sorption isotherms at 20°C for (MIX; *Corchous olitorius*) and (TOT 4157; *C. capsularis*) by equilibrating seeds over non-saturated LiCl solutions at around 40, 50, 60 and 70% relative humidity, and identified the RH at 20°C that yields an equilibrium moisture content equivalent to that attained at a standard accelerated ageing benchmark (45°C/ 60%RH). Linear interpolation across 20°C isotherms identified *Corchorus olitorius* accession MIX (59.71%) and *C. capsularis* accession TOT 4157 (59.83%) RH at 20°C as the equivalent moisture environment that yields the same equilibrium moisture content as 45°C/ 60% RH. Three accessions of each species were then equilibrated at the resultant eRH/ 20°C for each species, transferred for 24 hours to 45°C/ 60% RH, then sealed at 45°C and withdrawn for germination tests across 120 days. *C. olitorius* accessions were shorter-lived (58.9–84 days) than those of *C. capsularis* accessions (52.2–112.1 days). The slightly higher moisture affinity observed in *C. olitorius* is likely associated with increased rates of deterioration, unlike that of *C. capsularis*. The findings highlight the central role of precise moisture-temperature management in maintaining postharvest quality of jute mallow germplasm, providing guidance for genebank management, cold-chain storage and commercial applications.

Introduction

Jute mallow (*Corchorus* spp.) is an important crop which serves both as a vegetable and fibre crop and is cultivated in many parts of Africa and Asia (Ngomuo et al. 2017; Isuosuo et al. 2019). There are over 100 species belonging to the genus *Corchorus*, yet only two species, *C. olitorius* and *C. capsularis*, are commercialized (Zhang et al. 2019). As an opportunity crop, jute mallow is characterized by the limited development relative to their potential (N'Danikou et al. 2024a; Sileshi et al. 2025), thus, it has a poorly developed value chain. As a vegetable, jute mallow contributes significantly to nutrition security, especially in many regions of sub-Saharan Africa (Van Zonneveld et al. 2023). Beyond its role in traditional diets, jute mallow is also cultivated for bast fibre production, mainly in Asia forming the backbone of the global fibre industry after cotton (Del Río et al. 2009; Zhang et al. 2019). Despite this focal dual economic importance, the continued conservation and use of jute mallow are critically dependent on understanding its storage longevity, which remains a major limitation under ambient tropical conditions.

As jute mallow and crop wild related-species genetic resources are increasingly conserved in ex situ collections, the cost of maintaining viability through periodic regeneration cycles becomes a predominant operational concern (Balasupramaniyam et al. 2025). FAO Genebank Standards for orthodox seeds specify equilibration in a controlled condition of 5–20°C and 10–25% relative humidity, followed by storage at -18°C ± 3 (base collection) and 5 ± 3°C (active collection), with viability monitoring scheduled at intervals informed by species-specific longevity (FAO 2014, 2022; Hay et al. 2022b). For

most underutilized crops, including jute mallow, those intervals are extrapolated from a handful of model species (Kaweya et al. 2026; N'Danikou et al. 2024b; Shango et al. 2026; Van Zonneveld et al. 2021, 2023) and rarely reflect intra-specific variability. Comparative longevity phenotyping addresses this gap by ranking accessions on a deliberately short experimental timescale, using elevated temperature and relative humidity to accelerate ageing while preserving the rank order that would be observed under genebank conditions (Hay et al. 2022a; Balasupramaniyam et al. 2025). However, the use of these storage conditions for wild species and understudies crops such as jute mallow is still debatable (Hay and Probert 2013; Ghamkhar et al. 2025; Hay et al. 2025)

The most widely used comparative deterioration regimes, formalized by the Royal Botanic Gardens Kew (Balasupramaniyam et al. 2025; SER/INSR/RBGK 2023; Newton et al. 2014), holds equilibrated seeds at 45°C and 60% RH inside sealed foil pouches and tracks germination decline over time. The framework draws on the viability equation by Ellis and Roberts (1980a, b), which models the decline of probit-transformed germination as a linear function of storage time, with intercept (K_i) and slope ($1/\sigma$). The parameter sigma (σ), the period required for viability to drop by one probit, captures the temperature-moisture sensitive component of longevity and is therefore the quantity of greatest interest for genebank planning (Wolkis et al. 2025; Klepka et al. 2025).

A critical, sometimes overlooked, prerequisite for comparative deterioration is that all samples should enter storage at the same MC (Hay et al. 2022a; Mavi and Demir 2007; Newton et al. 2014). This may be attributed by the binding of water molecules to seed macromolecules weakens at higher temperatures, as the seeds equilibrated at a higher temperature reach a lower MC than the same cohort equilibrated a lower temperature (Hay et al. 2008, 2022). Hay et al. (2025) and Wijnker et al. (2025) highlights the importance of developing species-specific regeneration protocols and post-harvest practices to avoid unnecessary costs and most importantly the risk of genetic drift of the stored accessions in genebanks.

Despite the clarity of the species importance and framework, published longevity data for jute mallow are scant. The two species evaluated here, *Corchorus olitorius* and *C. capsularis*, are widely held in the World Vegetable Center Genebanks, and their relative storability is not formally documented. We addressed this gap through a two-step experiment (i) we determined the equilibrium moisture environment at 20°C that matches MC achieved at 45°C/60% RH using one accession per species and (ii) we quantified the longevity of three accessions per species at the resultant MC using probit regression analysis. Our objectives were (1) to demonstrate the feasibility of an isotherm- aligned controlled deterioration condition (CDC) protocol for jute mallow (2) to compare the storage longevity of three accession each for *C. olitorius* and *C. capsularis* and (3) to quantify intra-specific variation that genebanks should consider when scheduling viability monitoring for jute mallow

Materials and Methods

Seed materials

Accessions of *Corchorus olitorius* and *C. capsularis* were sourced from the germplasm base collection (-18°C) at the Africa's Vegetable Genebank maintained by the World Vegetable Center (WorldVeg), Eastern and Southern Africa (ESA). Partial sorption isotherms were developed on two species representative baseline accessions (MIX and TOT 4157). Accessions used in this study were selected based on initial quality, which should be over 85% viability and have sufficient seed amount for the experiment. Following these criteria, three accessions were selected per species for the storage experiments (Table 1). The initial viability for the tested accessions was confirmed to be above 97% prior the experiments.

Table 1
List of the accessions used in the present study

S/N	Species	Accession name	Regeneration year	Storage years	Genebank code	Country of origin
1	*C. olitorius*	MIX*	2015	11	RVI000644	Tanzania
2	*C. olitorius*	ES	2016	10	RVI000642	Tanzania
3	*C. olitorius*	SUD 4	2015	11	RVI000675	Sudan
4	*C. capsularis*	TOT 4157*	2015	11	RVI002592	Vietnam
5	*C. capsularis*	TOT 6278	2017	09	RVI002609	Vietnam
6	*C. capsularis*	ALV MN 059	2011	15	RVI002659	Unknown

RVI = Regional Genebank Vegetable Introduction Number, * = accessions used to determine sorption isotherms.

Moisture Sorption Isotherm Experiment (Step 1)

Estimation of initial water activity and moisture content

Seed packets were withdrawn from − 18°C storage freezers and equilibrated at room temperature (21.7°C) before opening to avoid condensation for initial MC and water activity (a_w; expressed as equilibrium relative humidity, eRH, where eRH = a_w × 100) determination. The seeds of jute mallow are known to have physical dormancy (Ibrahim et al. 2013). Thus, the samples were mechanically scarified using sandpaper (silicon carbide waterproof abrasive paper electrocoated) to break physical dormancy. Water activity was determined at 20°C (Hay et al. 2023), using a water activity station (HygroLab 3, Rotronic AG, Basserdorf, Switzerland). The moisture contents were determined gravimetrically, using the 'low constant temperature' oven method (ISTA 2023), drying samples of three replicates of 3–5 g seeds at 103°C for 17 ± 1 h. Upon removal from the oven, the seeds were allowed to cool for about 1 h in a

glass desiccator with silica gel at room temperature before re-weighing. Seed moisture contents are expressed on a fresh weight basis (% f.wt.) (Hay et al. 2023).

Determination of moisture sorption isotherms

Fifteen grams (15 g) of seed samples for each species were equilibrated at around 40, 50, 60 and 70% RH at 20°C using non-saturated lithium chloride ($LiCl_2$) solution in a glass desiccator for 14 days (Hay et al. 2008; Gómez Barreiro et al. 2025). The $LiCl_2$ solutions were prepared and kept at 20°C according to Hay et al. (2008), a week before the start of the experiments. Another 9 g of seeds of each species were equilibrated at 60% RH and 45°C, using another non-saturated $LiCl_2$ solution preparation described by Hay et al. (2022a)d mez Barreiro et al. (2025), and were left to equilibrate for a week at a constant 45°C. After equilibration, measurements of water activity and moisture content were performed.

Seed Storage Experiment (Step 2)

A non-saturated $LiCl_2$ solution was prepared for the second experiment following the same procedure described above (Step 1) for the equilibration of seeds at 20°C and a separate $LiCl_2$ solution prepared at 60% RH and 45°C (Hay et al. 2022a). After equilibration of seeds at 20°C for 14 days, the samples were placed at 60% RH and 45°C for 1 day (24 h) following Hay et al. (2022a), yielding 10.43% and 10.23% MC for *C. olitorius* (MIX) and *C. capsularis* (TOT 4157), respectively. Afterwards, each seed sample (≈ 0.23 g) was hermetically sealed in a laminated aluminium foil pouch (12 packets per accession, each with 100 seeds) and placed in an incubator at 45°C for accelerated ageing (AA). Seed storage experiment was determined using standard protocol (Hay et al. 2022a; Newton et al. 2014). For each accession, the 12 packets of seeds were removed from the experimental storage at predetermined intervals of 0, 1, 3, 5, 9, 20, 30, 40, 50, 60, 75 and 120 days. An additional 6 packets of 3 replicates for each species' accessions were made for gravimetric moisture content determination at the outset and the end of the experiment.

Seed germination testing

Seed germination was assessed using four replicates of 25 seeds per accession (ISTA 2023). The seeds were sown on wet tissue papers (top-of-paper method) with 6 ml of distilled water per Petri dish. They were incubated at a constant 20°C under an 8 h light/12 h dark cycle (Memmert IPP750 Plus, Memmert GmbH + Co. KG, Schwabach, Germany). Germination was recorded at 5, 7 and 14 days, with seeds considered germinated when the radicle had emerged by at least 2 mm (ISTA 2023).

Statistical analysis

All analyses were carried out in R statistical software (version 4.5.3; R Core Team 2025). The isotherm data were analysed in a restricted 40–60% RH range using linear regression (with 70% RH omitted) since the range of water activity used in this study represented a linear portion of the isotherms (relevant to

genebank storage conditions) and the available RH data were insufficient to fit a full isotherm model (Hay et al. 2022; Vijayan et al. 2025). Model fit was assessed using the coefficient of determination (R^2) and the significance of the regression slope. Seed survival data (ability of seeds to germinate after a given period during storage) were fitted by probit regression analysis (Ellis and Roberts 1980a), thereby fitting the seed viability equation to estimate the period for viability to fall to 50% (p_{50}), K_i and sigma (σ) (Eq. 1). Also, the squared Pearson correlation (R^2) between observed and predicted germination proportions by the model was performed. Following Wolkis et al. (2025), probit analysis was carried out fitting full models and reduced models in which survival curves were constrained into first, an independent, a common intercept (K_i) then common slope (1/σ) and lastly, a common line (common intercept and slope). Approximate tests (F-test; $P < 0.05$) were used to compare models and determine the best fit to accept. The analyses were carried out using R codes from Wolkis et al. (2025). Plots and summaries were built using packages '*ggplot2*' and '*dplyr*' in R software (Wickham et al. 2023).

$v = K_i - p/\sigma$ ..... [1]

where $\boldsymbol{\sigma}$ is the standard deviation of the frequency distribution of seed deaths in time, K_i is the probit viability of the seed lot at the beginning of storage. Estimates of time for viability to fall to 50% (p_{50}) were used as the measure of seed longevity using Eq. 2.

$p_{50} = K_i \times \sigma$ ..... [2]

Results

Moisture sorption characteristics of jute mallow accessions

A linear increase in seed moisture content with rising eRH was observed at 20°C for both species, especially over the limited 40–60% RH range (specifically targeting genebank storage conditions) (Table 2). In the analysis, we omitted 70% RH data points, but were shown in the plots thus the results are based on 40, 50 and 60% RH (Fig. 1). Correspondingly, a_w increased from approximately 0.43 to 0.70 in both species, confirming a strong positive relationship between MC and a_w. The relationship between MC and RH was nonlinear only when 70% RH included, but linear in the restricted 40–60% RH range with a steeper increase observed at higher RH levels, consistent with the typical sigmoidal orthodox moisture sorption behaviour (Fig. 1a, b). For *C. capsularis* (TOT 4157), MC rose from 8.2% at 40% RH to 12.61% at 70% RH, while *C. olitorius* (MIX) showed a similar trend, increasing from 8.33% to 13.2% across the same RH range (Table 2). At 60% RH and 45°C, accession MIX of *C. olitorius* produced an estimate of 10.43% moisture content and 59.71% eRH when projected onto 20°C. In contrast, the estimates for accession TOT 4157 of *C. capsularis* was 10.23% MC and 59.83% RH at the junction point of both 20°C and 45°C (Table 3). Temperature had a measurable effect on equilibrium MC (Fig. 1a, b). At 60% RH, increasing

temperature from 20°C to 45°C resulted in a slight reduction in MC in both species accessions. In TOT 4157, MC decreased from 10.55% to 10.23%, while in MIX, it decreased from 10.91% to 10.42% (Table 2).

Table 2
Equilibrium moisture content (eMC), equilibrium relative humidity (eRH) and water activity (a_w) of *C. olitorius* (MIX) and *C. capsularis* (TOT4157) accessions at 20°C and 45°C across different RH levels. Values are presented in means and standard deviation in parentheses.

Species	Accession	T (°C)	RH (%)	MC (%; f.wt)	eRH (%)	Water activity (a_w)
C. olitorius	MIX	20	40	8.33 (0.05)	44.07 (0.2)	0.44
			50	9.49 (0.04)	53.07 (0.42)	0.53
			60	10.91 (0.03)	63.17 (0.31)	0.63
			70	13.20 (0.24)	70.97 (0.49)	0.71
		45	60	10.42 (0.16)	61.87 (0.54)	0.62
C. capsularis	TOT 4157	20	40	8.20 (0.02)	43.11 (0.67)	0.43
			50	9.26 (0.05)	51.96 (0.29)	0.52
			60	10.55 (0.04)	62.46 (0.5)	0.62
			70	12.61 (0.14)	69.98 (0.39)	0.69
		45	60	10.23 (0.11)	62.2 (0.25)	0.62

Water activity standard error was so small that why weren't included (< 0.000)

The intercept and slopes of the experimental isotherm showed a slight difference between the two species accessions (Table 3). The fitted linear sorption parameters showed *C. olitorius* (MIX) exhibited a slightly lower intercept (2.34) than *C. capsularis* (TOT4157) (2.97), indicating a weaker inherent affinity for MIX moisture at lower water activities as compared to the latter. In contrast, the slope accession MIX of *C. olitorius* was slightly higher (0.14) compared to TOT 4157 of *C. capsularis* (0.12), indicating a greater change in seed moisture content with increasing relative humidity. The linear regression between eRH and MC at the 40–60% RH range showed a strong positive correlation ($R^2 > 0.99$) for both species, with a statistical significance ($p < 0.05$) relationship.

Table 3
Linear regression parameters of seed moisture content on equilibrium relative humidity in *C. olitorius* and *C. capsularis* at 20°C and 40, 50 and 60% RH. 70% RH was left out in the analysis. Standard error (s.e.) indicated in parentheses

Species	Accession	eRH (%)	MC (%; f.wt.)	Intercept	Slope	R^2	*P*
C. olitorius	MIX	59.71	10.43 (0.03)	2.34 (0.18)	0.14 (0.00)	0.99	0.01
C. capsularis	TOT 4157	59.83	10.23 (0.01)	2.96 (0.05)	0.12 (0.00)	1	0.02

Prediction of seed longevity

Linear mixed-effect model and Two One-Sided t-tests (TOST; $p < 0.01$) confirmed that the moisture content did not differ significantly ($p > 0.05$) between the first (0 – day) and last sampling (120 – day) times for these storage experiments, for all of the seed samples were equilibrated in the same environment (Table 4). Each treatment replicate was an independent aluminium foil pouch to minimize the inflation of significance and therefore a single t-test pooling across all accessions would conflates the variation hence the mixed model used. Seed survival curves for *Corchorus olitorius* accessions and *C. capsularis* accessions followed the expected negative cumulative normal distribution (Fig. 2) (Ellis 2022). Within species, fitting Eq. 1, it was possible to accept a common estimate for $1/\sigma$ among accessions for only *C. olitorius* species accessions without a significant increase in residual deviance (F-statistical probability < 0.05, Table 6), indicating that longevity is accession-dependent. However, K_i did not differ significantly among *C. capsularis* accessions ($p > 0.05$), suggesting uniform seed quality at the onset of storage. Also, significant differences in $1/\sigma$ ($p < 0.05$) revealed variation in deterioration rates, indicating that longevity is accession-dependent. The rejection of one-line models further confirms that seed survival trajectories cannot be generalized across accessions. Both K_i and $1/\sigma$ did not show a significant difference between the tested species accessions when common intercept and common slope were constrained against an independent model (F-statistical probability > 0.05; Table 7).

Table 4
Equilibrium moisture content of jute mallow accessions at conditioning (day 0) and after 120 days of storage from sorption isotherm projection. Values are mean and standard deviation (in parentheses) across three replicates.

Species	Accession	Initial MC (% f.wt)	Final MC (% f.wt)	MC Change (% f.wt)	Target (% f.wt)	Reached *p-value*	Held *p-value*
C. olitorius	ES	10.45(0.07)	10.43(0.07)	-0.02(0.12)	10.43	0.004	0.037
	MIX	10.44(0.12)	10.49(0.13)	0.05(0.14)		0.009	0.02
	SUD 4	10.44(0.07)	10.45(0.03)	0.02(0.04)		0.003	0.005
C. capsularis	ALV MN 059	10.22(0.10)	10.2(0.04)	-0.02(0.06)	10.23	0.009	0.014
	TOT 4157	10.25(0.08)	10.24(0.06)	-0.01(0.08)		0.008	0.017
	TOT 6278	10.16(0.13)	10.22(0.10)	0.06(0.04)		0.015	0.001

Reached target reports the larger of two one-sided t-test (TOST) p-values comparing day 0 MC to the derived sorption target and *Held target* reports the larger TOST p-value comparing day 0 to day 120 MC in storage.

Seed longevity (p_{50}) varied within both *C. olitorius and C. capsularis* species accessions but did not vary between the tested *C. olitorius and C. capsularis* species accessions (Tables 6 and 7). All accessions

noticeably maintained high viability before showing a progressive loss of germination over time (Fig. 2). For example, TOT 6278 noticeably showed a superior longevity (112 days) as compared to the rest of the accessions tested in both *C. olitorius* and *C. capsularis* accessions. Also, ALV MN 059 showed a poor storage lifespan (52 days) as compared to the rest of the accessions (Table 4). Overall, *C. capsularis* accessions maintained higher germination throughout the storage period, whereas germination of *C. olitorius* accessions declined slightly more rapidly (Table 4, Fig. 2).

Corchorus olitorius (a) and *Corchorus capsularis* (b). Each species was stored at different MCs

(10.43% and 10.23% MC for *C. olitorius* and *C. capsularis*, respectively).

Table 5
Estimated parameters of the survival curves for *Corchorus olitorius* (10.43% MC) and *C. capsularis* (10.23% MC) with standard error (s.e.) of the estimates in parentheses (from fitted curves shown in Fig. 2)

Species	**Accession**	K_i **(NED)**	**1/(σ) (NED; day^{-1})**	**Sigma (σ; days)**	p_{50} **(days)**	R^2
C. olitorius	ES	3.06(0.32)	-0.04(0.00)	26.9(3.37)	82.2 (4.12)	0.99
	MIX	2.27(0.22)	-0.04(0.00)	25.93(2.87)	58.9 (3.18)	0.97
	SUD4	2.17(0.19)	-0.03(0.00)	38.7(4.59)	84 (5.50)	0.98
C. capsularis	ALV MN 059	1.93(0.18)	-0.04(0.00)	26.93(2.86)	52.2 (2.99)	0.98
	TOT 4157	2.46(0.23)	-0.03(0.00)	32.96(3.84)	81.2 (4.76)	0.92
	TOT 6278	2.04(0.18)	-0.02(0.00)	55.06(8.39)	112.1 (10.57)	0.92

K_i represents the intercept (initial probit seed viability before experimental storage), sigma (σ)= standard deviation of seed death at a given time, slope or beta (1/σ) = sigma inverse or a direct measure of how quickly seed viability declines with larger σ indicates slow rate of viability loss and vice versa, p_{50} (half-life) = time for viability to decline to 50% widely used as a benchmark for comparing how long seeds remain viable, NED = normal equivalent deviates (probit units).

Table 6
Comparison of four nested models for seed survival curves using Eq. 1, among accessions within *Corchorus olitorius* and *Corchorus capsularis*, A for Model fits (Deviance table) and B for F-tests during storage at 45°C over 120 days

(A) Model fits	**Deviance**	**df**	**AIC**
Corchorus olitorius			
(1) Independent	15.907	30	98.635
(2) Common K_i	22.685	32	101.452
(3) Common σ	23.518	32	101.051
(4) One line	48.012	34	121.848
Corchorus capsularis			
(1) Independent	31.585	30	121.233
(2) Common K_i	35.254	32	120.102
(3) Common σ	49.677	32	134.743
(4) One line	102.15	34	180.467
(B) F-tests for nested model comparisons	Δ df	*F*	*P*
Corchorus olitorius			
(2) Common K_i vs (1) Independent	2	6.391	0.005
(3) Common σ vs (1) Independent	2	7.177	0.003
(4) One line vs (2) Common K_i	2	17.864	< 0.001
(4) One line vs (3) Common σ	2	16.664	< 0.001
Corchorus capsularis			
(2) Common K_i vs (1) Independent	2	1.742	0.192
(3) Common σ vs (1) Independent	2	8.592	0.001
(4) One line vs (2) Common K_i	2	30.361	< 0.001
(4) One line vs (3) Common σ	2	16.901	< 0.001

AIC = Akaike Information Criterion, *F* = F-statistics, Δ df = change in degree of freedom

Table 7
Comparison of four nested models in Eq. 1, for seed survival curves between *Corchorus olitorius* and *Corchorus capsularis* during storage at 45°C over 120 days.

(A) Model	**Deviance**	**df**	**AIC**	**Δ df**	***F***	***P***
(1) Independent	150.162	68	302.315			
(2) Common K_i	154.770	69	304.041			
(3) Common σ	154.681	69	304.255			
(4) One line	155.115	70	302.466			
(B) F-tests model comparisons						
(2) Common K_i vs (1) Independent				1	2.087	0.153
(3) Common σ vs (1) Independent				1	2.046	0.157
(4) One line vs (2) Common K_i				1	0.154	0.696
(4) One line vs (3) Common σ				1	0.194	0.661

Discussion

Our two-step findings deliver a moisture-matched comparison of seed longevity of *C. olitorius* and *C. capsularis* accessions consistent with FAO Genebank standards framework and with the comparative deterioration approach standardized by the Millenium Seed Bank Partnership (FAO 2014, 2022; Hay et al. 2022a; Newton et al. 2014). The use of non-saturated LiCl solutions allowed us to set precise eRH targets in both 20°C and 45°C environments, while the linear regression of MC against eRH within the restricted 40–60% RH (for genebank storage) window was sufficient to identify the equivalent equilibrium environment (59.71% and 59.83% RH at 20°C, for (MIX) *C. olitorius* and (TOT 4157) *C. capsularis*, respectively) without requiring the full isotherm model fits.

Temperature influenced the sorption profiles, with seeds exposed to 45°C stabilizing at lower MC compared to those at 20°C under similar RH conditions (Fig. 1a, b; Table 2). Moisture content decreased with increasing temperature at constant water activity (Fig. 1). This inverse relationship between temperature and equilibrium moisture content (EMC) aligns with the Clausius-Clapeyron principle and has been widely reported for orthodox seeds (Iglesias and Chirife 1976; Vertucci 1990; Vertucci and Leopold 1987). At an elevated temperature, the vapour pressure deficit between the seed surface and the surrounding air increases, thereby reducing the net moisture absorbed. For instance, at around 60% RH, both species exhibited a downward shift in EMC when held at 45°C, with the effect more pronounced in MIX of *C. olitorius* (Fig. 1a). Such findings emphasize the importance of considering both RH and temperature simultaneously when defining safe and acceptable storage conditions, or experimental seed storage conditions. The optimal conditions for seed storage are those that, from a thermodynamic or

kinetic perspective, minimize any deteriorative reactions (Vertucci 1990, 1993; Vertucci and Leopold 1987).

The moisture sorption behaviour of orthodox species typically follows a reverse sigmoidal trend (hygroscopic behaviour), where water is progressively adsorbed as environmental humidity rises (Hay et al. 2022b; Rezaei et al. 2023). In our experiment, specifically targeting genebank storage conditions, we used a few (target 40, 50 and 60 RH range), and therefore it wasn't possible to get a full reverse sigmoid-shaped curve (Fig. 1), but with an inclusion of 70% RH, the nonlinear pattern emerges. The phenomenon was also documented in Vijayan et al. (2025) where only the linear part of the isotherm was used and could therefore not be fitted the full isotherm equation. However, the seed moisture content increased progressively with rising RH as expected, and as reported in several orthodox species (Rosa 2021; Zhu et al. 2021; Rezaei et al. 2024). Hay et al. (2022) and Zhu et al. (2021) highlights that at a low RH, water is tightly bound to polar sites, while at higher RH levels, multilayer adsorption and capillary condensation contribute to the sharper increase in MC. At 20°C, both species accessions displayed equilibrium moisture content in the range of 8–13% across the tested RH levels, consistent with the reported hygroscopic nature of seeds (Table 2). The derived partial isotherms resembled the characteristic Type II curve with an inclusion of 70% RH as described by Hay et al. (2022b) and Rezaei et al. (2023).

A comparison of the two species accessions revealed subtle differences in moisture uptake at equivalent RH. At 59.71% RH, *C. olitorius* (MIX) reached a slightly higher level of 10.43% MC whereas *C. capsularis* (TOT 4157) equilibrated at approximately 10.23% MC at 59.83% RH (Fig. 1a, b). These differences may reflect inherent compositional variation between the species, likely in seed coat structure, lipid content and surface area available for water sorption. SER/INSR/RBGK (2023), an online seed information database (SID), reports a provisional oil content of 12.7% for *C. olitorius* and 13.2% for *C. capsularis*. Oil content generally affects moisture relations, but not in isolation (Demir et al. 2011; Zhu et al. 2021), which makes oil-rich seeds absorb less water at a given RH as compared to starchy seeds due to the hydrophobic nature of lipids (Jones and Earle 1966). Although the differences are small, they may have practical implications for seed storage behaviour, as higher MC at given RH can accelerate seed deterioration. Also, Vertucci (1993) reported that the way water binds within seeds and the impact of bound water on cellular physiology play a key role in shaping both the nature and kinetics of seed deterioration.

Seed longevity is a critical factor for both agriculture and biodiversity conservation. Seed longevity during storage differs widely across species, influenced in part by seed composition (Ellis and Hong 2007). It is further shaped by the combined effects of environmental conditions during maturation and harvest, the timing of harvest and post-harvest handling practices such as the duration and drying conditions, as well as pre-storage environment (Kochanek et al. 2009; Probert et al. 2009; De Vitis et al. 2020). When comparing the two species accessions, the tested *Corchorus* species did not show significant difference in performance ($p > 0.05$) (Table 7). The observed variation in seed longevity within ($p < 0.05$; Table 6) *Corchorus olitorius* and *C. capsularis* demonstrates the importance of the intra-specific differences in determining seed storage abilities. *C. olitorius* maintained higher viability during the early

storage phase before declining sharply beyond 50 days. The MIX accession deteriorated most rapidly (58.9 days), suggesting lower inherent longevity or a higher sensitivity to moisture-related ageing. In contrast, ES and SUD 4 retained viability for a longer duration, indicating a stronger physiological resilience (Table 5). The shallower slope of SUD 4 curves implies a lower rate constant for seed death, which is often associated with superior seed membrane integrity and enhanced repair mechanisms during storage (Walters 2015; Nagel et al. 2019). A similar trend was observed in *C. capsularis*, where all accessions maintained high viability up to approximately 25 days, followed by a progressive decline. Among these, ALV MN 059 exhibited the fastest loss of viability (52 days), probably due to the longest storage time (from 2011) while TOT 4157 (81.2 days) and TOT 6278 (112 days) demonstrated prolonged viability during storage (Table 5). The trend within the accessions of each species underscores the significance of intra-specific diversity for genebank management. For instance, *C. olitorius* (accession MIX) and *C. capsularis* (accession ALV MN 059) showed a rapid viability loss, suggesting they may require a quick and shorter regeneration cycles or stricter storage protocols. In contrast, *C. olitorius* (SUD 4) and *C. capsularis* (TOT 6278) exhibited greater longevity and could be prioritized for long-term conservation, as they are more likely to survive longer under conventional genebank storage conditions. These differences underscore a considerable intra-specific variation (Fig. 2) in seed longevity, potentially influenced by seed coat properties, ecotypes, lipid composition and antioxidant defence systems. The intraspecific variation observed is large enough to matter for genebank management as genebank curators routinely schedule viability monitoring interval using species-level averages, yet the present data suggest that accessions of the same species may require different monitoring intervals. Thus, where resources permit, accession-specific longevity estimates derived from this study can be embedded into the viability monitoring schedule, increasing operational efficiency and reducing the risk of late detection of viability loss for jute mallow germplasm.

The limitation of present study includes the focus on limited accessions per species and limited range of RH as we mainly targeted useful genebank conditions. The study used only three levels of RH (40, 50 and 60%) to capture the partial moisture sorption behaviour for MIX and TOT 4157 accessions as representatives of *C. olitorius* and *C. capsularis* in a linear part of the isotherms which is of the core interest for genebanks. The increase in MC and a_w at higher RH levels is critical because seed deterioration rates increase rapidly above certain thresholds (typically $a_w > 0.6$). In this study, both species accessions approached a_w values of 0.7 at 70% RH, which may place seeds risk of accelerated ageing, fungal growth and overall reduced viability i.e we omitted 70% RH in the analysis as it is of little or no interest in genebank management. Therefore, extending the present study comparison to a wide panel of jute germplasm, multiple harvesting seasons and natural ageing environment would clarify how robust the *C. olitorius* and *C. capsularis* contrast is across genetic and environmental background. The variation in seed longevity within *Corchorus* species accessions is attributed to many interactive effects, ranging from genetics, seed development, how seeds are processed, and the length of time before storage and to date we know very little about this interaction with storage lifespan of seeds (Merritt et al. 2014; White et al. 2023). Also, its critical to note that predictions of seed longevity should be interpreted with caution as estimates are extrapolated from seed viability models (Hay et al. 2014; De Vitis et al.

2020; Kaweya et al. 2026; Shango et al. 2026), and particularly in the context of long-term storage as seed lots used aren't from the same regeneration year. The present study nonetheless provides a defensible baseline against which future *Corchorus* longevity phenotyping can be calibrated.

Conclusion

The two-step experiment aligned the moisture content at 45°C/60% RH with an equivalent equilibration RH at 20°C where 59.71% RH; *C. olitorius* (MIX) and 59.83% RH; *Corchorus capsularis* (TOT 4157) is feasible and reproducible for the two species. Comparative controlled deterioration showed that *C. capsularis* accessions outlived *C. olitorius* accessions on average, with significant detectable intra-specific variation that warrants accession-specific viability monitoring schedules. The also study highlights the higher moisture affinity observed in MIX of *C. olitorius* tracks its faster deterioration, indicating that sorption behaviour can be used as a candidate proxy for longevity screening in jute mallow germplasm. These findings indicate that accession and species-specific moisture relations should be considered when developing storage protocols for *Corchorus* germplasm, and for wild species and other traditional African vegetables, where the use of conventional practices of other crops remains prominent.

Declarations

Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contribution

AS. S.N. E.W.M. conceived the paper AS. E.W.M. S.N. wrote the methodology S.N. H.D.M. P.M.K. provided supervision S.N. provided resources E.W.M. A.S. collected the data E.W.M curated the data and formal

analysis E.W.M. wrote the original draft E.W.M., A.S., S.N., H.D.M., P.M.K. writing, review, and editing. All authors have read and approved the final version of the manuscript.

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Data Availability

The findings of this study are available from World Vegetable Center, but restrictions apply to the availability of these data, which were used under licence for the current study and so are not publicly available. The data are, however, available upon request and with the permission of the World Vegetable Center and corresponding author.

References

1. Balasupramaniyam S, Merritt DJ, Hay FR, Dalziell EL (2025) Assessing the storage potential of seed collections to inform the management of wild species seed banks. Aust J Bot 73(1). https://doi.org/10.1071/BT24065
2. De Vitis M, Hay FR, Dickie JB, Trivedi C, Choi J, Fiegener R (2020) Seed storage: maintaining seed viability and vigor for restoration use. Restor Ecol 28(S3). https://doi.org/10.1111/rec.13174
3. Del Río JC, Rencoret J, Marques G, Li J, Gellerstedt G, Jiménez-Barbero J, Martínez ÁT, Gutiérrez A (2009) Structural Characterization of the Lignin from Jute (*Corchorus capsularis*) Fibers. J Agric Food Chem 57(21):10271–10281. https://doi.org/10.1021/jf900815x
4. Demir I, Kenanoglu BB, Hay F, Mavi K, Celikkol T (2011) Determination of seed moisture constants (K E, C W) for the viability equation for watermelon, melon, and cucumber seeds. Seed Sci Technol 39(2):527–532. https://doi.org/10.15258/sst.2011.39.2.23
5. Ellis RH, Hong TD (2007) Seed longevity – moisture content relationships in hermetic and open storage. Seed Sci Technol 35(2):423–431. https://doi.org/10.15258/sst.2007.35.2.17
6. Ellis RH, Roberts EH (1980a) Improved Equations for the Prediction of Seed Longevity. Ann Bot 45(1):13–30. https://doi.org/10.1093/oxfordjournals.aob.a085797
7. Ellis RH, Roberts EH (1980b) The Influence of Temperature and Moisture on Seed Viability Period in Barley (Hordeum distichum L.). Ann Bot 45(1):31–37. https://doi.org/10.1093/oxfordjournals.aob.a085798

8. FAO (2016) Genebank standards for plant genetic resources for food and agriculture (Revised edition).
9. FAO (2022) Practical guide for the application of the Genebank Standards for Plant Genetic Resources for Food and Agriculture: Conservation of orthodox seeds in seed genebanks. FAO
10. Ghamkhar K, Hay FR, Engbers M, Dempewolf H, Schurr U (2025) Realizing the potential of plant genetic resources: the use of phenomics for genebanks. PLANTS PEOPLE PLANET 7(1):23–32. https://doi.org/10.1002/ppp3.10570
11. Gómez Barreiro P, Hudson AR, Hay FR, Adams J (2025) *LiCl Calc*: tools to use lithium chloride solutions to control relative humidity in seed experiments. Seed Sci Technol. https://doi.org/10.15258/sst.2025.53.2.08
12. Hay FR, Adams J, Manger K, Probert R (2008) The use of non-saturated lithium chloride solutions for experimental control of seed water content. Seed Sci Technol 36(3):737–746. https://doi.org/10.15258/sst.2008.36.3.23
13. Hay FR, Baum (Née Whitehouse) KJ, Oyatomi O, Wolkis D (2025) Optimizing the accession-level quantity of seeds to put into storage to minimize seed (gene)bank regeneration or re-collection. Conserv Physiol 13(1):coaf011. https://doi.org/10.1093/conphys/coaf011
14. Hay FR, Davies RM, Dickie JB, Merritt DJ, Wolkis DM (2022a) More on seed longevity phenotyping. Seed Sci Res 32(3):144–149. https://doi.org/10.1017/S0960258522000034
15. Hay FR, Mead A, Bloomberg M (2014) Modelling seed germination in response to continuous variables: use and limitations of probit analysis and alternative approaches. Seed Sci Res 24(3):165–186. https://doi.org/10.1017/S096025851400021X
16. Hay FR, Probert RJ (2013) Advances in seed conservation of wild plant species: a review of recent research. Conserv Physiol 1(1):cot030–cot030. https://doi.org/10.1093/conphys/cot030
17. Hay FR, Rezaei S, Buitink J (2022b) Seed Moisture Isotherms, Sorption Models, and Longevity. Front Plant Sci 13:891913. https://doi.org/10.3389/fpls.2022.891913
18. Hay FR, Rezaei S, Wolkis D, McGill C (2023) Determination and Control of Seed Moisture. Seed Sci Technol 51(2):267–285. https://doi.org/10.15258/sst.2023.51.2.11
19. Ibrahim H, Oladiran JA, Mohammed H (2013) Effects of seed dormancy level and storage container on seed longevity and seedling vigour of jute mallow (Corchorus olitorius). Afr J Agric Res 8(16):1370–1374. https://doi.org/10.5897/AJAR12.1839
20. Iglesias HA, Chirife J (1976) A model for describing the water sorption behavior of foods. J Food Sci 41(5):984–992. https://doi.org/10.1111/j.1365-2621.1976.tb14373.x
21. ISTA (2023) International Rules for Seed Testing, International Seed Testing Association. Bassersdorf, Switzerland
22. Isuosuo CC, Akaneme FI, Abu NE (2019) Nutritional Evaluation of the Seeds of Corchorus olitorius: A Neglected and Underutilized Species in Nigeria. Pak J Nutr 18(7):692–703. https://doi.org/10.3923/pjn.2019.692.703

23. Jones Q, Earle FR (1966) Chemical analyses of seeds II: Oil and protein content of 759 species. Econ Bot 20(2):127–155. https://doi.org/10.1007/BF02904010
24. Kaweya BS, N'Danikou S, Shango AJ, Sanga HG, Kabululu MS, Hamisy W, Tryphone GM, Van Zonneveld M (2026) Derivation of seed viability constants (K_E and C_W) and prediction of seed longevity for spider plant (*Cleome gynandra*). Seed Sci Technol. https://doi.org/10.15258/sst.2026.54.1.03
25. Klepka L, Liepelt S, Bucharova A (2025) Large interspecific and intraspecific variation in seed longevity in storage
26. Kochanek J, Steadman KJ, Probert RJ, Adkins SW (2009) Variation in seed longevity among different populations, species and genera found in collections from wild Australian plants. Aust J Bot 57(2):123. https://doi.org/10.1071/BT09023
27. Mavi K, Demir I (2007) Controlled deterioration and accelerated ageing tests to predict seedling emergence of watermelon under stressful conditions and seed longevity. Seed Sci Technol 35(2):445–459. https://doi.org/10.15258/sst.2007.35.2.19
28. Merritt DJ, Martyn AJ, Ainsley P, Young RE, Seed LU, Thorpe M, Hay FR, Commander LE, Shackelford N, Offord CA, Dixon KW, Probert RJ (2014) A continental-scale study of seed lifespan in experimental storage examining seed, plant, and environmental traits associated with longevity. Biodivers Conserv 23(5):1081–1104. https://doi.org/10.1007/s10531-014-0641-6
29. Nagel M, Seal CE, Colville L, Rodenstein A, Un S, Richter J, Pritchard HW, Börner A, Kranner I (2019) Wheat seed ageing viewed through the cellular redox environment and changes in pH. Free Radic Res 53(6):641–654. https://doi.org/10.1080/10715762.2019.1620226
30. N'Danikou S, Ayenan MAT, Sigalla JP, Shango AJ, Zonneveld MV (2024a) Storage behaviour and seed longevity of traditional African vegetables: a review. Seed Sci Technol 52(2):177–192. https://doi.org/10.15258/sst.2024.52.2.04
31. N'Danikou S, Nickas J, Shango AJ, Kilasi N (2024b) Derivation of seed viability constants (*KE* and *CW*) and prediction of longevity of *Solanum aethiopicum* seeds. Seed Sci Technol 52(2):193–203. https://doi.org/10.15258/sst.2024.52.2.05
32. Ngomuo MS, Stoilova T, Feyissa T, Kassim N, Ndakidemi PA (2017) Leaf and seed yield of jute mallow (*Corchorus olitorius* L.) accessions under field conditions for two consecutive growing seasons. J Hortic Sci Biotechnol 92(6):614–620. https://doi.org/10.1080/14620316.2017.1304168
33. Newton R, Hay FR, Probert RJ (2014) Protocol for comparative seed longevity testing. Technical Information Sheet, 1, Royal Botanic Gardens Kew, Richmond, UK https://doi.org/10.13140/RG.2.2.19429.81129
34. Probert RJ, Daws MI, Hay FR (2009) Ecological correlates of ex situ seed longevity: a comparative study on 195 species. Ann Bot 104(1):57–69. https://doi.org/10.1093/aob/mcp082
35. R Core Team (2025) R: A Language and Environment for Statistical Computing. R foundation for statistical computing: Vienna, Austria. Available from: <https://www.R-project.org%3E (Accessed 20 March 2026).

36. Rezaei S, Buitink J, Hay FR (2023) Contrasting seed moisture sorption behaviour between two species and the implication for seed longevity. Seed Sci Res 33(2):126–134. https://doi.org/10.1017/S0960258523000156
37. Rezaei S, Buitink J, Hay FR (2024) Seed responses to change in ambient humidity and the consequences for storability. J Stored Prod Res 109:102477. https://doi.org/10.1016/j.jspr.2024.102477
38. Rosa DP (2021) Water sorption properties of papaya seeds (*Carica papaya* L.) formosa variety: An assessment under storage and drying conditions
39. Shango AJ, Mosha EW, N'Danikou S, Ramadhani S, Kamala M, Sumaye S, Venkataramana P, Mtui H, Philipo M, Kusolwa P, Van Zonneveld M (2026) Derivation of seed viability constants (C_W, K_E) and predicting seed longevity for jute mallow (*Corchorus capsularis* and *C. olitorius*). Seed Sci Technol. https://doi.org/10.15258/sst.2026.54.2.01
40. Sileshi GW, Borgemeister C, Kuyah S, Nath AJ, Arshad S (2025) About Neglected and underutilized crops for agroecological transition of food systems. CABI Agric Biosci:0047. https://doi.org/10.1079/ab.2025.0047
41. SER/INSR/RBGK (2023) *Seed Information Database (SID)*, Society for Ecological Restoration, International Network for Seed-Based Restoration, Royal Botanic Gardens Kew. Available from: %3Chttps://ser-sid.org/%3E (Accessed 25 March 2026).
42. Van Zonneveld M, Kindt R, McMullin S, Achigan-Dako EG, N'Danikou S, Hsieh W, Lin Y, Dawson IK (2023) Forgotten food crops in sub-Saharan Africa for healthy diets in a changing climate. Proc Natl Acad Sci 120(14):e2205794120. https://doi.org/10.1073/pnas.2205794120
43. Van Zonneveld M, Kindt R, Solberg SØ, N'Danikou S, Dawson IK (2021) Diversity and conservation of traditional African vegetables: Priorities for action. Divers Distrib 27(2):216–232. https://doi.org/10.1111/ddi.13188
44. Vertucci CW (1990) Calorimetric studies of the state of water in seed tissues. Biophys J 58(6):1463–1471. https://doi.org/10.1016/S0006-3495(90)82491-7
45. Vertucci CW (1993) Predicting the optimum storage conditions for seeds using thermodynamic principles
46. Vertucci CW, Leopold AC (1987) The Relationship between Water Binding and Desiccation Tolerance in Tissues. Plant Physiol 85(1):232–238. https://doi.org/10.1104/pp.85.1.232
47. Vijayan SS, Rezaei S, Hay FR (2025) Water activity-moisture content relations of primed, film-coated and/or pelleted vegetable seeds. Seed Sci Technol. https://doi.org/10.15258/sst.2025.53.2.05
48. Walters C (2015) Orthodoxy, recalcitrance and in-between: describing variation in seed storage characteristics using threshold responses to water loss. Planta 242(2):397–406. https://doi.org/10.1007/s00425-015-2312-6
49. White FJ, Hay FR, Abeli T, Mondoni A (2023) Two decades of climate change alters seed longevity in an alpine herb: implications for ex situ seed conservation. Alp Bot 133(1):11–20. https://doi.org/10.1007/s00035-022-00289-8

50. Wijnker E, Bouchaut D, Van Treuren R, Van Hintum T (2025) A pragmatic protocol for seed viability monitoring in ex situ plant genebanks. Genet Resour Crop Evol 72(1):49–59. https://doi.org/10.1007/s10722-024-02019-x
51. Wolkis D, Carta A, Rezaei S, Hay FR (2025) Seed longevity: analysing post-storage germination data in R to fit the viability equation. Seed Sci Res 35(1):33–40. https://doi.org/10.1017/S0960258524000291
52. Zhang L, Ibrahim AK, Niyitanga S, Zhang L, Qi J (2019) Jute (*Corchorus* spp.) Breeding. In: Al-Khayri JM, Jain SM, Johnson DV (eds) Advances in Plant Breeding Strategies: Industrial and Food Crops. Springer International Publishing, Cham, pp 85–113
53. Zhu G, 1. College of Engineering, China Agricultural University, Beijing 100083, China, Jin Q, Liu Y, Lin Y, Wang J, Li X, 2. School of Pharmacy, Gannan Medical University, Ganzhou 341000, Jiangxi, China (2021) Moisture sorption and thermodynamic properties of Camellia oleifera seeds as influenced by oil content. Int J Agric Biol Eng 14(1):251–258. https://doi.org/10.25165/j.ijabe.20211401.5457

Figures

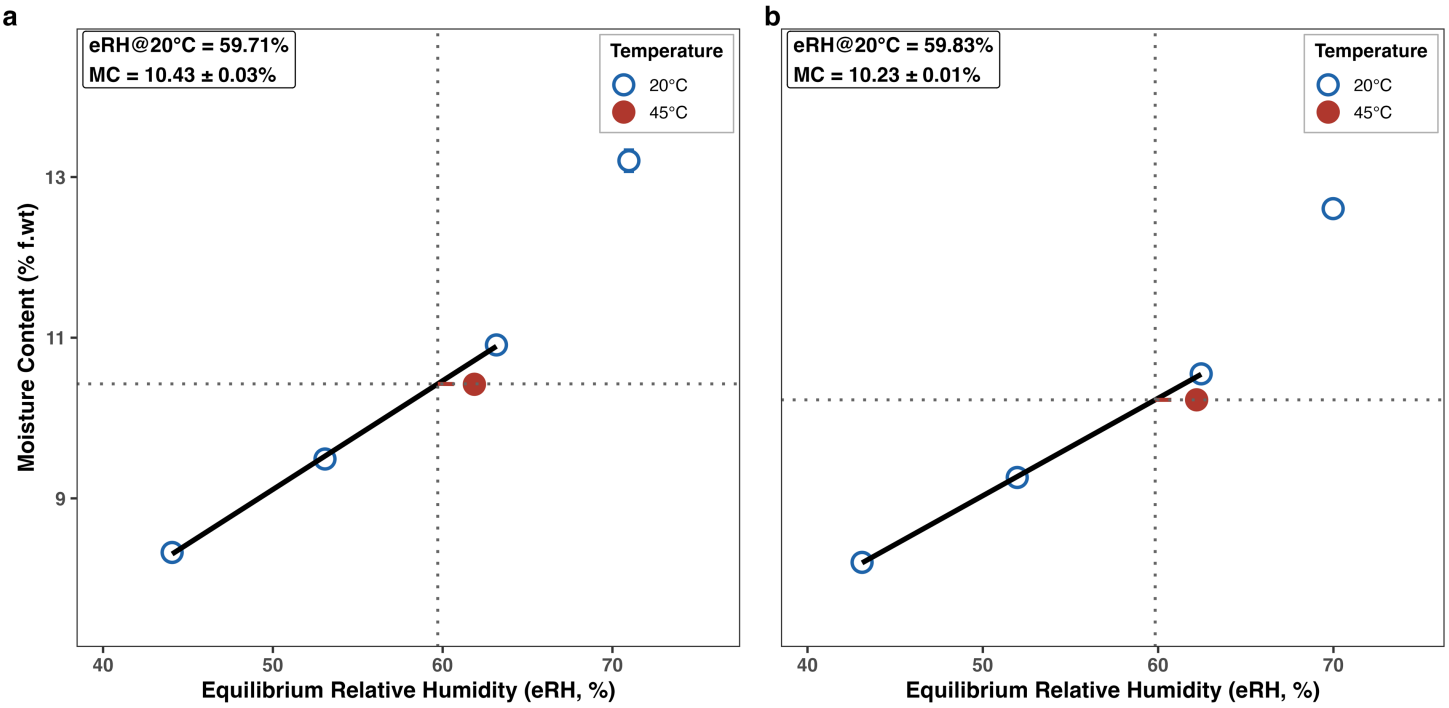

Figure 1

Relationship between eRH and seed moisture content for (a) *Corchorus olitorius* (accession MIX) and (b) *C. capsularis* (accession TOT 4157) at 20°C and 45°C. The data are represented in means and standard error. The black straight line represents the linear regression of data collected at 40, 50 and 60% and 20°C (hollow-blue circles), while red filled circle points indicate data collected at 60% and 45°C. The horizontal dashed-line connects the 45°C value to the 20°C regression, and the vertical dotted line

indicates the estimated eRH at 20°C required to reach the same MC. The round blue-hollow circle that is left out of others, indicate data collected at 70% RH, at 20°C, which was omitted in the analysis.

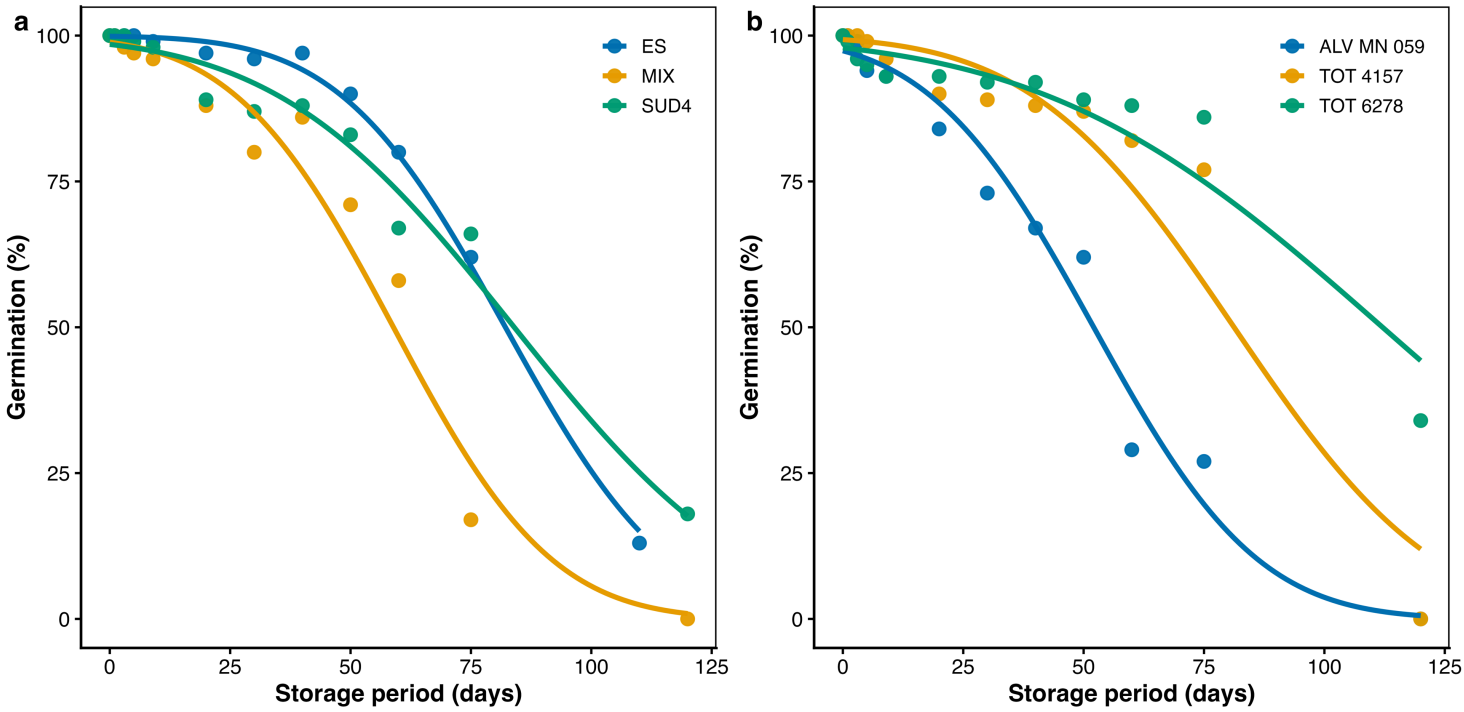

Figure 2

Survival curves with the accessions for each species at 45°C for 120 days of storage

Corchorus olitorius (a) and *Corchorus capsularis* (b). Each species was stored at different MCs

(10.43% and 10.23% MC for *C. olitorius*and *C. capsularis*, respectively).