

Research Paper

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Dry storage alters intraspecific variation in phenotypic traits at early life stages: evidence from a dominant alpine meadow species

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Abstract

The intraspecific variations of phenotypic traits in the early life stages, such as seed germination and seedling establishment, are important components affecting species adaptation and differentiation. As one of the most common ways in which seeds are maintained, dry storage usually affects these traits, by either increasing or decreasing variation among populations. However, little is known about how the interaction between dry storage and population variation of a species affects the performance and adaptability of early life-cycle traits. In this study, we conducted experiments with seeds from ten populations of *Elymus nutans* along an altitudinal gradient on the eastern Qinghai-Tibet Plateau. Fresh seeds and seeds stored dry for 6 months were used to quantify the temperature thresholds for germination, determine seedling emergence and survival in two common gardens and examine the correlation between these traits and environmental conditions of population provenance. Dry storage increased germination percentage, germination speed and seedling emergence, and decreased intraspecific variation in germination traits of seeds (reduced by 33.36, 52.05 and 20.45% for T_b , $\theta_{T(50)}$ and $\sigma_{\theta T}$ respectively). Dry storage had little effect on the intraspecific variation of seedling emergence and survival. In addition, the temperature threshold for germination cannot be used to predict seedling emergence and survival in either common garden, regardless of whether seeds were stored or not. These results indicated that it is feasible to use dry-stored seeds to evaluate and select suitable provenances in ecological restoration, and using dry-stored seeds in ecological restoration projects is expected to achieve better vegetation restoration results than fresh seeds. On the other hand, the status of seeds (fresh vs. dry stored) should be considered in an evaluation of the adaptive value of plant functional traits, especially in the early life stages, otherwise, inconsistent conclusions may be drawn.

Introduction

Early life stages of plants, including seed germination and seedling establishment, are critical phases in their life cycle (De Frenne et al., 2012). These stages often exhibit variation among populations due to disparities in habitat conditions (Cochrane et al., 2015; Cochrane, 2016; Ramirez-Valiente et al., 2021). Acquiring comprehensive insights into the influence of environmental factors on seed germination and seedling establishment at the population level is vital for understanding the ecological adaptation of species in the context of climate change. Furthermore, it is pivotal for the development and implementation of effective restoration strategies (Fenner and Thompson, 2005; Walck et al., 2011; Vazquez-Ramirez and Venn, 2021).

Generally, intraspecific variation in environmental requirements for seed germination is affected by genetics and the habitat conditions where the population is established (Baskin and Baskin, 2014; Lortie and Hierro, 2021). Ahmed and Escobar-Gutiérrez (2022) have reported that the optimum temperature for germination of *Dactylis glomerata* seeds from a low-altitude population was higher than that of a high-altitude population. However, there have also been studies (Del Vecchio et al., 2012; Santo et al., 2014) that found the temperature requirement for seed germination is independent of the habitat. An important reason for this controversy is that the germination traits are not only dependent on the genetic background but also on the environmental conditions seeds experienced before and after dispersal. For example, dry storage has been found to affect seed dormancy release, which consequently alters germination characteristics and associated functional traits, especially for species whose seeds have non-deep physiological dormancy (Baskin and Baskin, 2014; Zhang et al., 2017). Picciau et al. (2019) have shown that dry storage at 25°C for 3 months alters the

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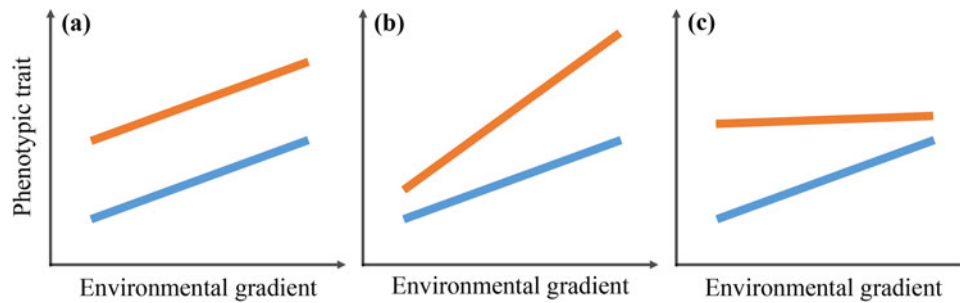


Figure 1. A conceptual model: reaction norm curves of phenotypic traits for fresh and stored seeds to environmental gradient. The solid blue and orange lines represent the reaction norm curves for fresh and stored seeds, respectively. These curves demonstrate the increasing trend of a specific trait along an environmental gradient, such as altitude, longitude, latitude, etc. The left graph indicates that the impact of dry storage on phenotypic traits does not alter their intraspecific variation (a). On the other hand, the middle and right graphs depict that the effect of dry storage is population-dependent, leading to an increase (b) or decrease (c) in the intraspecific variation of traits.

response of seed germination to temperature in different *Helichrysum microphyllum* populations. Although many studies have concluded that dry storage can alter germination traits, it is not clear how this effect changes intraspecific variation and post-germination performance. For example, the effect of dry storage or seed afterripening on phenotypic traits may be independent of the population, indicating that dry storage does not affect the intraspecific variation of traits (Fig. 1a). On the other hand, the ability of seeds to after-ripen may be related to the population, thus increasing (Fig. 1b) or decreasing (Fig. 1c) the intraspecific variation of phenotypic traits. Therefore, it is worth clarifying whether dry storage interacts with the population and thus affects the evaluation of the adaptation value of seed germination traits.

Similarly, the capacity of seedling emergence and survival under field conditions could display significant population variability (López et al., 2019), and this variation is largely related to the germination characteristics of seeds (Walck et al., 2011). Generally, the time of seed germination is closely related to the time of seedling emergence in the field, which may affect seedling survival (Baskin and Baskin, 2014). Considering that dormancy-break during dry storage affects the germination characteristics of seeds, it is reasonable to assume that dry storage of seeds also alters seedling emergence and survival in the field. However, it has not been determined if dry storage affects intraspecific variation in seedling phenotypic traits. Numerous studies have shown that phenotypic traits at the seedling stages vary among populations (Barton et al., 2020; Lortie and Hierro, 2021). For example, seeds of *Erysimum capitatum* tend to germinate to higher percentages in their native habitat than in a foreign habitat; however, high seedling survival was observed only in high-altitude populations (Blanquart et al., 2013). Gonzalo-Turpin and Hazard (2009) found that whether seedling survival of *Festuca eskia* has an adaptive advantage in its native habitat is related to seed provenance. Therefore, our hypothesis is that dry storage not only affects seed germination traits, seedling emergence and survival, but it also leads to changes in the intraspecific variation of these traits.

Moreover, from the perspective of ecological restoration, it is crucial to find a simple and easy method to select the provenance(s) of seeds to be used in the field. Therefore, there is an increasing interest in predicting the performance of seedlings in the field through seed germination traits in the laboratory (Kolasinska et al., 2000; Zhang et al., 2021). However, the performance of seedlings is often affected by many factors. For

example, a high germination percentage and germination speed may be beneficial for plants to occupy growing space and improve their competitiveness in a relatively stable environment (Jorritsma-Wienk et al., 2007). However, in highly unpredictable environments, dormancy or delayed germination is often more beneficial to improve the fitness of populations to the environment (Donohue et al., 2010). Thus, the time of seed germination will largely determine the establishment of seedlings (Limon and Peco, 2016). This implies that predicting seedling establishment in the field based on seed germination traits is likely to be a potential candidate model. However, Baldini et al. (2018) have pointed out that seed pre-treatment (cold stratification) can affect the relationship between lab germination percentage and field seedling emergence, indicating that this relationship largely depends on the dormancy status of seeds. Considering that dry storage can break dormancy, it can affect germination characteristics and thus alter the emergence phenotype under field conditions. Therefore, we hypothesize that dry storage may impact the relationship between lab germination traits and field seedling performance. However, it should be noted that there is still limited evidence from relevant experiments.

Elymus nutans (Poaceae) is a perennial grass and is one of the dominant species of alpine meadow on the Qinghai-Tibet Plateau, mainly distributed in the altitude range of 2500–4000 m a.s.l (Liu et al., 2014). Due to its wide distribution range, this species is a very suitable research organism to clarify the intraspecific variation of functional traits on early life stages under global climate change. To test our hypotheses, we collected seeds of *E. nutans* along an altitude gradient on the eastern Qinghai-Tibet Plateau and conducted laboratory germination tests and field experiments in two common gardens using fresh and stored seeds. We expected to find variation in germination traits and seedling performance among populations and addressed the following questions: (1) Does dry storage affect intraspecific variation of early life-cycle traits? (2) Do the results from germination tests in the laboratory relate to seedling establishment in the field? If so, is this relationship affected by dry storage?

Materials and methods

Seed collection

Freshly matured seeds from ten populations of *E. nutans* were collected in September 2016 along an altitudinal gradient on the eastern Qinghai-Tibet Plateau (Gansu province, China) (Fig. 2).

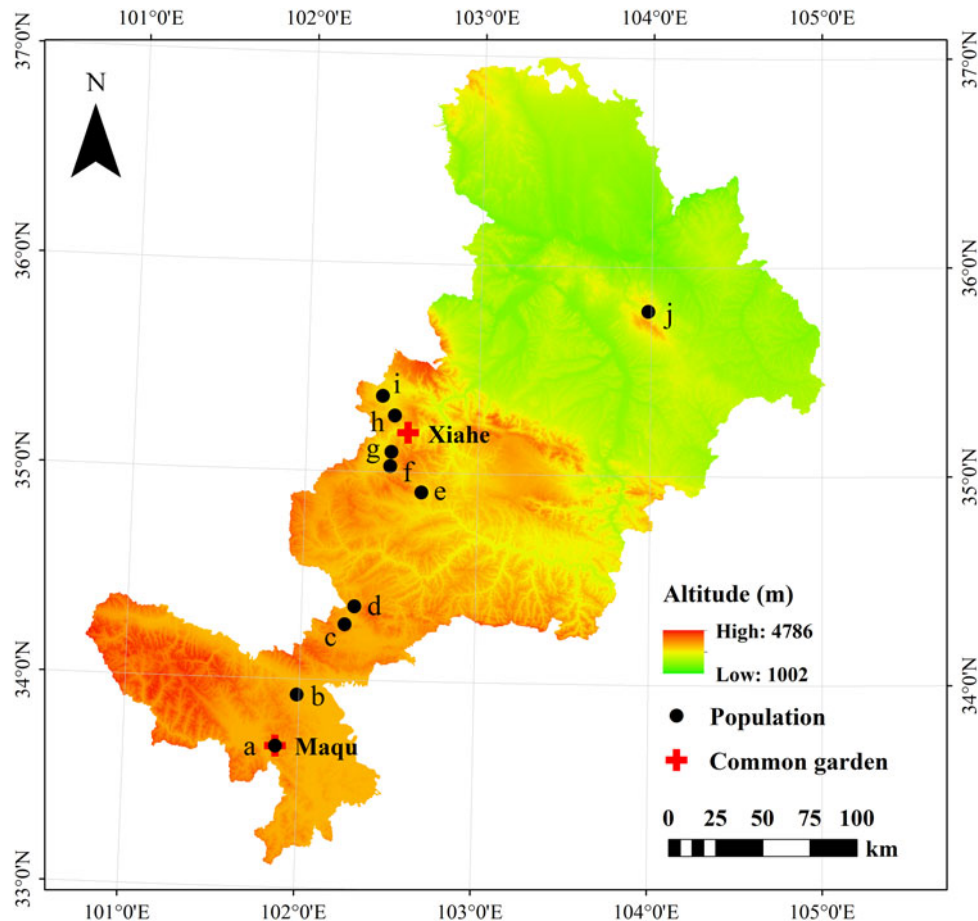


Figure 2. Locations of seed collection sites and common garden sites.

Infructescences with ripe seeds were collected from several hundred individual plants at each of the ten collection sites and taken to the laboratory, where seeds were separated from other plant material. Seeds were dried at room temperature for 1 week (RH 20–35%, 18–25°C) and stored at –20°C until used in experiments. Germination tests and field experiments for fresh seeds were conducted within 2 weeks after seed collection.

Laboratory germination experiments

Germination responses to temperature were tested for fresh seeds from all populations by incubation at 10, 15, 20 and 25°C in light (12/12 h). Photon irradiance was $60 \mu\text{mol m}^{-2} \text{s}^{-1}$, 400–700 nm, from white fluorescent tubes. For each test, three replicates of 50 seeds were placed in 10 cm Petri dishes on two sheets of filter paper (Shuangquan, Hangzhou, China) moistened with 7 ml distilled water. All Petri dishes were placed in the incubator and distilled water was added daily as needed to keep the filter paper moist. Germination was monitored daily for at least 28 days until no further germination occurred for three consecutive days. Seeds were counted as germinated when the radicle was visible (≥ 2 mm).

To determine the effect of dry storage on seed germination, fresh seeds from each of the ten populations were placed in paper bags and stored at room temperature (RH 20–35%, 18–25°C) for 6 months. After dry storage, germination was tested at 10, 15, 20 and 25°C in light as described earlier.

Common garden experiments

To determine the effect of population, growing site, dry storage and their interactions on seedling establishment, both fresh and stored (RH 20–35%, 18–25°C, 6 months) seeds from seven (a, b, f, g, h, i and j) out of the ten populations were sown at Xiahe (35°11' N, 102°35' E, 2900 m a.s.l, hereafter XH) and Maqu (34°41' N, 101°52' E, 3500 m a.s.l, hereafter MQ) on the Plateau. Fresh seeds were sown in October 2016, and stored seeds were sown in April 2017. XH has a Mean annual temperature (MAT) and mean annual precipitation (MAP) of 2.6°C and 516 mm, respectively, and for MQ 1.2°C and 620 mm, respectively.

The field experiment was performed in a completely randomized block design. Twenty replicates per population and 20 seeds per replicate were used in this experiment. A total of 11,200 seeds was used in common garden experiments (2 common gardens \times 7 populations \times 20 replicates \times 20 seeds \times 2 seed states). To facilitate the identification of emerging seeds, we prepared the ground by placing iron fences with a grid size of 5 \times 5 cm and securing them with u-nails before sowing. For each seed population, the seeds were sown (one seed per each 5 \times 5 cm area) at a depth of 1 cm with a spacing between each sowing of 20 cm. Seedling emergence and survival were monitored every 2 weeks for 5 months during the growing season. A seedling was counted as emerged when the true leaves were visible, and a seedling was classified as surviving if it remained without withering or

dying after emerging from the ground. The emergence percentage was calculated by dividing the number of seeds that have emerged by the number of seeds sown, while the survival percentage was determined by dividing the number of seedlings that survived by the number of seedlings that emerged. During the growing season, weeds were manually removed at 2-week intervals without applying any herbicide treatment.

Soil sampling and chemical measurements

Three soil samples were collected from each of the 10 sites in 2016. Each sample was comprised of five soil cores (0–10 cm depth) taken with a soil auger (7.5 cm inner diameter). All soil samples were placed in individual plastic bags and immediately stored in a portable refrigerator. In the laboratory, all soil samples were subdivided into two subsamples. One subsample was stored at 4°C until analysis for NO₃⁻-N (NON) and NH₄⁺-N (NHN). The second subsample was air-dried at room temperature and used for analysing available phosphorus (AP). To determine NON and NHN, a subsample of 10 g fresh soil was extracted in 50 ml of a 2M KCl solution and then analysed by colorimetry (UV-1601, Shimadzu Inc.). AP was extracted in 50 ml of a 0.5 M NaHCO₃ solution (pH = 8.5) from 5 g dry soil subsample, and then the amount was measured calorimetrically using the molybdate-ascorbic acid method UV-1601, Shimadzu Inc.).

Climate data

Climate data were obtained from the Loess Plateau SubCenter, National Earth System Science Data Center, National Science and Technology Infrastructure of China (<http://loess.geodata.cn>) (Peng et al., 2019). MAT and MAP from 2000 to 2020 were used to characterize the climatic variations at the ten seed collection sites. MAT and MAP data for the ten collection sites were extracted through ArcGIS.

Data analysis

Generalized linear mixed models (GLMMs) were performed, using the *glmer* function in the *lme4* package of R version 4.1.2 to investigate the effect of storage, population, temperature and their interactions on germination percentage and germination speed ($1/t_g$). Similarly, the effect of common garden, storage, population and their interactions on seedling emergence and seedling survival were tested by fitting GLMMs. In each model, these variables were used as fixed effects, while replicates were included as random effects. Seed germination and seedling establishment were a probability ranging from 0 to 1; hence, we applied a binomial estimation of the model using a logit link function. Duncan's test was used to compare means when significant differences were found.

Germination speed was represented by $1/t_g$ (the reciprocal of t_g), and t_g is defined as the time to reach a given germination fraction g . t_g was estimated using a GERMINATOR package developed by Joosen et al. (2010) using the visual basic module from Microsoft Excel.

A thermal time model was used to quantify the temperature requirements at suboptimal temperature ranges for seed germination according to Bradford (2002). Germination percentage response can be expressed as:

$$\theta_{T(g)} = (T - T_b) \cdot t_g$$

and the relationship among germination percentage, imbibition time and temperature is:

$$\text{probit}(g) = \frac{\ln((T - T_b) \cdot t_g) - \ln(\theta_{T(50)})}{\sigma_{\ln\theta T}}$$

where $\theta_{T(g)}$ is the thermal time required to reach a given germination fraction g (with units of °C per day or hour); T is the germination temperature; T_b is the base temperature below which a seed will not germinate; t_g is the actual time to germination of fraction g ; $\text{probit}(g)$ is the probit transformation of cumulative germination percentage g , which linearizes the sigmoidal time course on a log time scale (Finney 1971); $\theta_{T(50)}$ and $\sigma_{\ln\theta T}$ are the median thermal time and standard deviation of $\ln(\theta_T)$ requirements among individual seeds in the population, respectively. The thermal time model was fitted to data using non-linear regression in SPSS 25.0 (SPSS Inc., Chicago, IL). Goodness-of-fit between predicted and observed data was assessed by the coefficient of determination (R^2).

$$R^2 = 1 - \frac{\sum (y_{\text{obs}} - y_{\text{pre}})^2}{\sum (y_{\text{obs}} - \bar{y}_{\text{obs}})^2}$$

where y_{obs} is the observed value, y_{pre} is the predicted value, \bar{y}_{obs} is the mean of the observed value.

General linear regression using the *lm* function in the *stats* package of R version 4.1.2 was performed to test how increasing altitude where seeds were collected affected T_b , $\theta_{T(50)}$, seedling emergence and seedling survival, both in fresh and stored seeds.

Redundancy analysis (RDA) using the *rda* function in the *vegan* package of R version 4.1.2 were used to examine the relationships between seed germination traits and environmental conditions where the seeds matured. Additionally, we investigated the relationship between seed germination traits and seedling traits, such as emergence and survival. The significance of these relationships was assessed using Monte Carlo permutation test. We checked for multicollinearity of explanatory variables before RDA analysis and excluded variables with a variance inflation factor (VIF) greater than 10. Therefore, T_b and $\theta_{T(50)}$ were included as response variables, while MAT, MAP, NON, NHN and AP as explanatory variables. Before implementing the RDA process, the analysed data were normalized based on Euclidean distance (Warton et al., 2012).

Results

Geographical variation in seed germination

Storage, population, temperature and their interactions had significant effects on the germination percentage of *E. nutans*, and temperature, storage × temperature, population × temperature and storage × population × temperature significantly affected germination speed ($P < 0.001$) (Table 1). Germination percentage and speed of all populations increased as temperature increased, regardless of whether seeds were stored or not (Supplementary Fig. S1, S2). The temperature requirements for seed germination varied with populations (Table 2).

For fresh seeds, the altitude where seeds were collected was negatively correlated with T_b ($R = -0.766$, $P = 0.010$), but it was not correlated with $\theta_{T(50)}$ ($P = 0.552$, data not shown) (Fig. 3). The RDA showed that environmental factors explained 84.90%

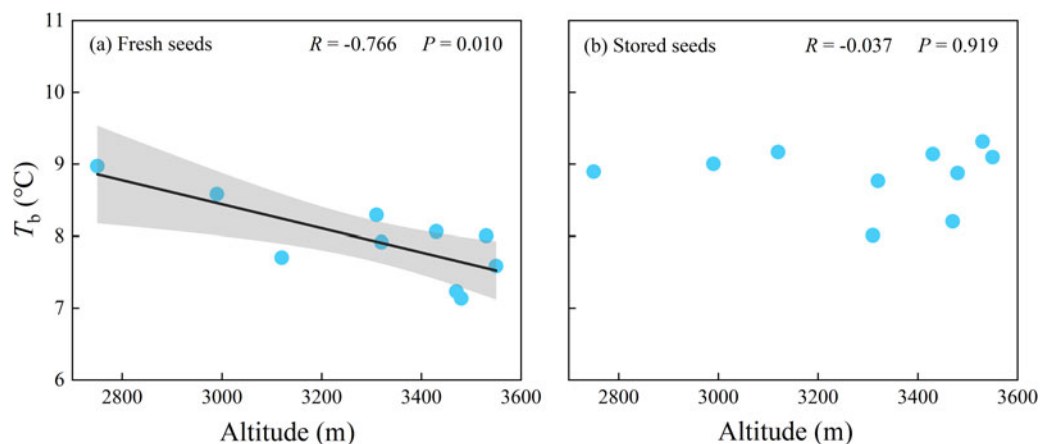
Table 1. Effect of storage, population, temperature and their interactions on germination percentage and speed of *E. nutans* seeds using generalized linear mixed models.

Source	Germination percentage			Germination speed		
	Chisq	Df	P	Chisq	Df	P
Storage (S)	15.29	1	<0.001	0.00	1	1.000
Population (P)	102.95	9	<0.001	0.00	9	1.000
Temperature (T)	284.90	3	<0.001	49.09	3	<0.001
S * P	82.47	9	<0.001	3.38	9	0.947
S * T	17.33	3	<0.001	72.76	3	<0.001
P * T	175.26	27	<0.001	918.98	27	<0.001
S * P * T	107.91	27	<0.001	181.74	27	<0.001

Table 2. Germination parameters of fresh and stored seeds (6-month storage) of *E. nutans* populations based on the thermal time model.

Population	Suboptimal temperature							
	Fresh				Storage			
	T_b	$\theta_{T(50)}$	$\sigma_{\ln\theta T}$	R^2	T_b	$\theta_{T(50)}$	$\sigma_{\ln\theta T}$	R^2
A	8.01	120.88	1.02	0.91	9.31	32.47	1.11	0.91
B	7.23	150.83	1.09	0.90	8.20	51.71	0.98	0.87
C	7.14	129.70	0.97	0.68	8.87	37.04	0.82	0.84
D	7.59	213.73	1.07	0.64	9.10	33.73	1.12	0.90
E	8.07	276.55	1.36	0.70	9.14	47.34	1.03	0.91
F	7.92	286.13	1.22	0.66	8.77	36.99	0.92	0.88
G	7.70	176.16	1.00	0.72	9.17	37.24	0.93	0.87
H	8.29	128.38	1.23	0.88	8.01	39.60	0.82	0.83
I	8.58	197.16	1.16	0.68	9.00	42.22	0.84	0.85
J	8.97	102.67	1.50	0.44	8.89	29.72	0.90	0.81
Average	7.95	178.22	1.16	-	8.85	38.81	0.95	-
CV (%)	7.19	36.23	14.79	-	4.79	17.37	11.77	-

T_b , base temperature; $\theta_{T(50)}$, thermal time for 50% of seeds to germinate at suboptimal temperature ranges; $\sigma_{\ln\theta T}$, standard deviation for $\ln(\theta_T)$; R^2 , coefficient of determination; CV, coefficient of variation.

**Figure 3.** The relationships between the altitude and base temperature for fresh and stored seeds. The correlation coefficient (R) and P values are shown in each figure. The fitted lines are from OLS regression. Significant relationships ($P < 0.05$) are denoted with solid lines, and grey areas indicate the 95% confidence interval of the fit.

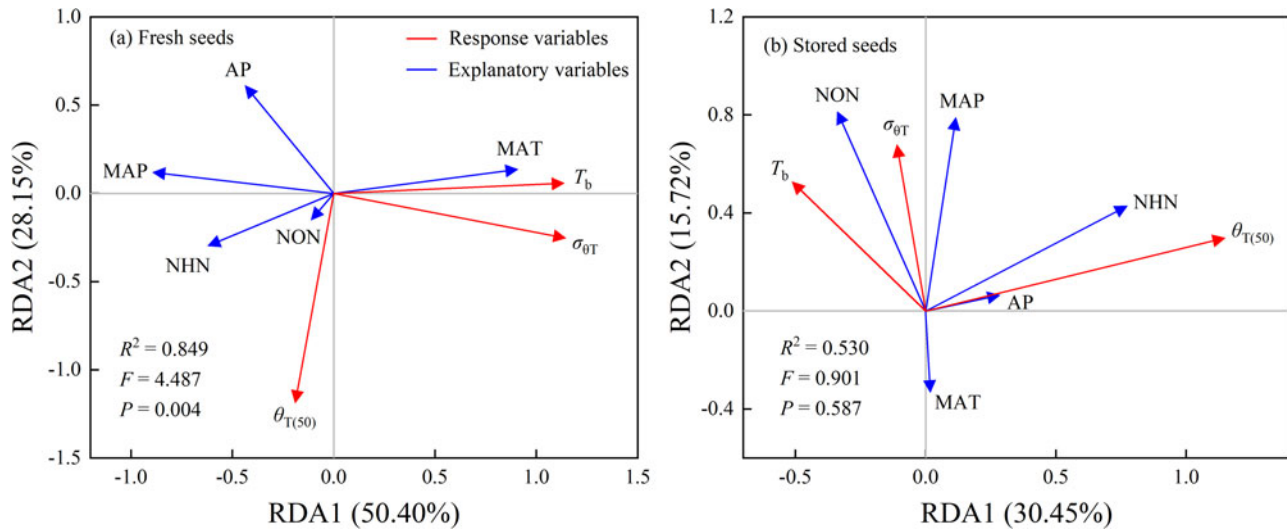


Figure 4. The results of redundancy analysis (RDA) between environmental conditions and temperature thresholds for germination of fresh and stored seeds. Red and blue arrows indicate response and explanatory variables, respectively. T_b , base temperature; $\theta_{T(50)}$, thermal time for 50% of seeds to germinate at suboptimal temperature ranges; $\sigma_{\ln\theta_T}$, standard deviation for $\ln(\theta_T)$; MAT, mean annual temperature; MAP, mean annual precipitation; NON, NO_3^- -N; NHN, NH_4^+ -N; AP, available phosphorus.

of the variance in temperature thresholds for germination. The Monte Carlo permutation test found that variance in temperature thresholds was significantly explained by the first two axes ($F = 4.487$, $P = 0.004$), with 50.40 and 28.15% for RDA1 and RDA2, respectively (Fig. 4a). However, altitude was independent of T_b ($P = 0.919$, data not shown) and $\theta_{T(50)}$ ($P = 0.435$, data not shown) for stored seeds. The Monte Carlo permutation test found that variance in temperature thresholds was not significantly explained by the environmental factors ($F = 0.901$, $P = 0.587$), with 30.45 and 15.72% for RDA1 and RDA2, respectively (Fig. 4b).

Six months of dry storage increased germination percentage and speed in all populations (Supplementary Fig. S1, S2). In addition, storage decreased $\theta_{T(50)}$ but increased T_b in all populations, except for populations *h* and *j*. For example, the mean values of T_b and $\theta_{T(50)}$ for fresh seeds were 7.95 and 178.22 °C·d, respectively. For stored seeds, the mean values were 8.85 and 38.8 °C·d, respectively (Table 2).

Seedling establishment in the field

Common garden, storage, population and their interactions had significant effects on both seedling emergence and survival ($P < 0.01$). However, it is worth noting that only storage did not exhibit a significant effect on seedling survival ($P = 0.226$) (Table 3). Seedling emergence and survival of all populations were higher in the high-altitude common garden (Maqu) than at the low altitude (Xiahe). Six months of dry storage significantly increased seedling emergence of *E. nutans* in both common gardens ($P < 0.05$), while the effect on seedling survival depended on the population (Fig. 5). In addition, the relationship between seedling emergence and survival with altitude depended on seed status and common garden. For fresh seeds, no correlations were found between the altitude of population and seedling emergence in either of the two common gardens. However, in the low-altitude common garden (Xiahe), there was a negative correlation between the altitude of seed collection and seedling survival for stored seeds ($R = -0.344$, $P < 0.001$) (Fig. 6).

Table 3. Effect of common garden, storage, population and their interactions on seedling emergence and survival of *E. nutans* using generalized linear mixed models.

Source	Seedling emergence			Seedling survival		
	Chisq	Df	<i>P</i>	Chisq	Df	<i>P</i>
Common garden (CG)	9.80	1	<0.001	155.81	1	<0.001
Storage (S)	62.93	1	<0.001	1.47	1	0.226
Population (P)	47.16	6	0.005	101.37	6	<0.001
CG * S	28.84	1	<0.001	11.10	1	<0.001
CG * P	20.64	6	0.002	19.07	6	0.004
S * P	36.50	6	<0.001	66.40	6	<0.001
CG * S * P	60.29	6	<0.001	49.26	6	<0.001

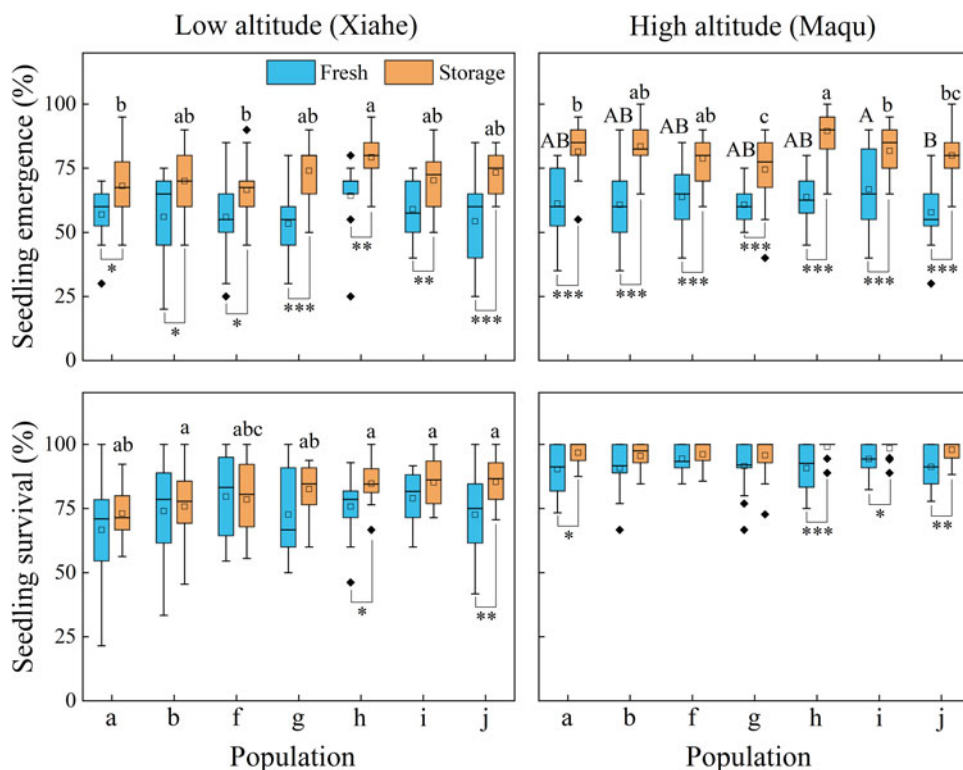


Figure 5. Seedling emergence and survival for fresh and stored seeds of seven *E. nutans* populations in both common gardens. Bars with different uppercase and lowercase letters indicate that significant differences in phenotypic traits among populations for fresh and stored seeds at the level of 0.05, respectively. *, ** and *** indicate significant effects of dry storage on phenotypic traits at the level of 0.05, 0.01 and 0.001, respectively.

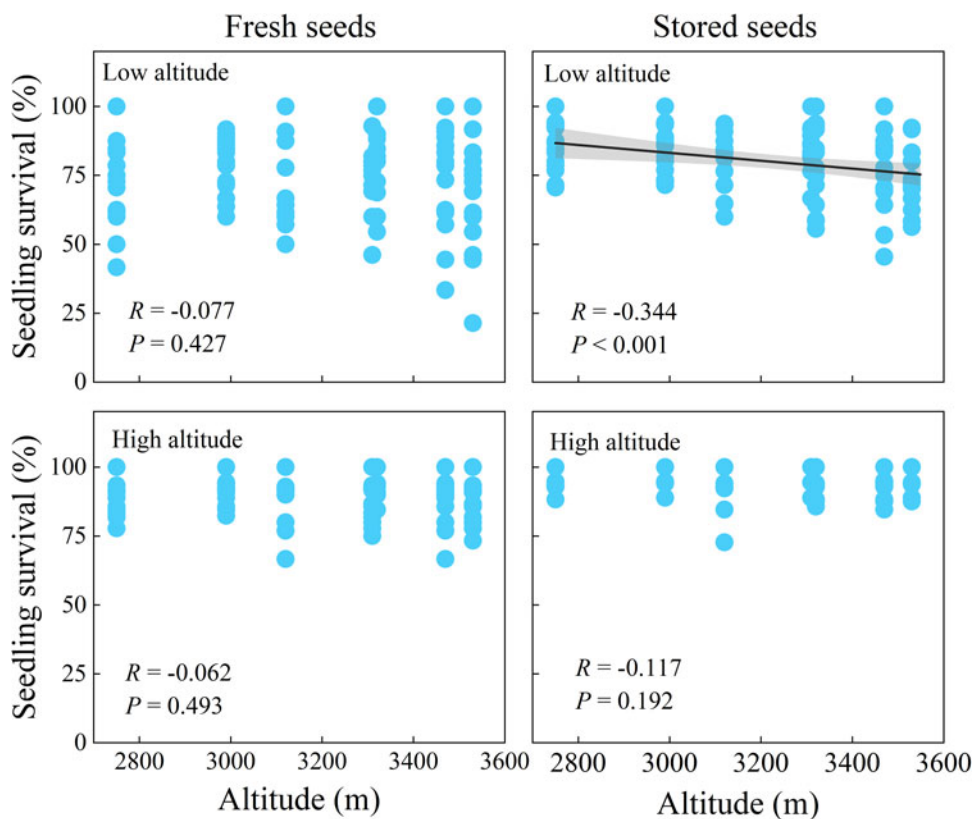


Figure 6. The relationships between the altitude and seedling emergence and survival for fresh and stored seeds. The correlation coefficient (*R*) and *P* values are shown in each figure. The fitted lines are from OLS regression. The solid black lines indicate significant relationships (*P* < 0.05), and grey areas indicate the 95% confidence interval of the fit.

Relationship between seed germination and seedling establishment

The Monte Carlo permutation test found that variance in seedling characteristics, including seedling emergence and survival, was not significantly explained by temperature threshold for germination in either common garden, regardless of whether seeds were stored or not (Supplementary Fig. S3).

Discussion

We found that intraspecific variation in early traits especially for temperature thresholds of seed germination is highly dependent on seed status (fresh vs. dry stored). Seed status not only affected the variation of these life-cycle traits among populations, but it also altered their relationship with the maternal environment, which supports our first hypotheses. Thus, we conclude that the estimation of intraspecific variation must take seed status into account. Contrary to our second hypothesis, seedling performance was not correlated with germination traits in either common garden, regardless of whether seeds were stored or not. Thus, the temperature for germination in the laboratory could not be used to predict seedling emergence and survival, at least in our study.

Dry storage altered intraspecific variation in seed germination and seedling establishment

Numerous studies have reported that dry storage significantly affects early life-cycle traits especially for species that have physiological dormancy (Jose et al., 2011; Baskin and Baskin, 2014; Zhang et al., 2017; Bhatt et al., 2020). Consistent with previous studies, we found that dry storage increased germination percentage and speed, indicating that at least a portion of the fresh seeds were dormant, which agrees with Chen et al. (2018) who reported that fresh seeds of *E. nutans* have non-deep physiological dormancy. Dry storage can significantly increase the germination percentage of seeds with non-deep physiological dormancy, such as *Paulownia elongata* (Liu et al., 2017) and *Silybum marianum* (Monemizadeh et al., 2021).

The influence of dry storage on seed germination traits is highly dependent on the population, thus further altering its intraspecific variation. Our results showed that dry storage decreased intraspecific variation in germination traits, in which the coefficient of variation of T_b , $\theta_{T(50)}$ and $\sigma_{\theta T}$ was reduced 33.36, 52.05 and 20.45%, respectively. Wang et al. (2009) reported that dry storage decreased the intraspecific variation in germination percentage and speed of *Berberis verna*. However, Zhang et al. (2017) found that dry storage increased among-population differences in germination percentage of *Stipa bungeana*. Thus, dry storage can play a role in generating intraspecific variation in seed germination traits, but this effect may be species-specific. The fact that dry storage can significantly affect intraspecific variation could result in under- or overestimation of the effect of population and thus lead to a biased conclusion when considering which seed provenance(s) to use for restoration. Although Baskin and Baskin (2014) strongly recommend using fresh seeds in seed ecological studies, one must be very cautious to control the external environment condition during and after seed collection in particular for those seeds with non-deep physiological dormancy. An alternative way to solve this problem is to wait for seeds experiencing the rapid transition stage, such as from dormant to non-deep physiologically dormant seeds. The downside

is that seeds after storage may not exactly represent the true conditions they experienced in the field. However, in many species with non-deep physiological dormant seed, seed dormancy will decrease very quickly even if exposed to the field conditions (Baskin and Baskin, 2014). The only difference between fresh and dry seeds is that the presence of short-term dormancy in fresh seeds may help to inhibit seedling emergence before winter, and then they act the same in the coming spring. Another effect is that fresh seeds can avoid germination immediately after dispersal, thus avoiding competition with the mother plant. Thus, if we expect to see how differences in germination traits affect seedling emergence in the coming year, using dry stored seeds may be preferred.

Consistent with germination traits, dry storage also significantly increased seedling emergence for all populations under field conditions. On the one hand, seed dormancy was broken during dry storage, which increased the germination percentage and further improved the emergence. On the other hand, although dormancy in fresh seeds can be broken while experiencing winter conditions (cold stratification) (Chen et al., 2021), this process may increase the risk of predation in the field, thereby reducing the seedling emergence. However, contrary to germination traits, dry storage had little effect on the intraspecific variation of seedling traits, especially in the low-altitude common garden (Xiahe). Thus, the effect of dry storage on seedling performance may be independent of population, which may have some beneficial implications for habitat restoration. That is, fresh or stored seeds can be selected according to different research purposes.

In addition, we found that seedling performance differed greatly between common gardens, with higher emergence and survival in the high altitude than in the low altitude common garden (Maqu), suggesting that seedling emergence and survival are largely dependent on the environmental conditions where seeds are sown. A longer, wetter and cooler chilling period in high than in the low altitude common garden, no doubt facilitates the dormancy release of *E. nutans* seeds, which increased seedling emergence (Chen et al., 2021). On the other hand, low soil water content during summer in low-altitude common garden was not conducive to seedling survival (Supplementary Fig. S4), resulting in relatively high mortality. Kim et al. (2013) showed that at a high altitude site more seedlings of *Erysimum capitatum* derived from seeds produced in high as compared to low altitude populations survived. In contrast, seedlings suffered higher mortality when they were planted at the low than at the high altitude site. These results suggest that the selection of sites in which to conduct common garden experiments may influence the assessment of seedling adaptation.

Relationship between seed germination and seedling establishment

Previous studies have shown that germination traits in the laboratory are useful in predicting seedling establishment in the field (Tobe et al., 2005; Rosbakh and Poschlod, 2015). Germination percentage of seeds was positively (Kolasinska et al., 2000) or negatively (Baldini et al., 2018) correlated with seedling establishment. However, Blossey et al. (2017) found that laboratory germination tests did not reflect seedling emergence of *Alliaria petiolata* under field conditions. Consistent with this, we found that the percentages of seedling emergence and survival were not correlated with the temperature threshold for germination,

regardless of study site and seed status. A possible reason is that the temperature and thermal time during the germination season in our study area are much higher than the T_b and $\theta_{T(50)}$ requirements for germination (Supplementary Fig. S5), indicating that temperature may not be a limiting factor for seedling emergence of *E. nutans*. These results indicate that the relationship between seedling establishment and germination traits can be very complex. Thus, seedling establishment is not only dependent on the ability of seeds to germinate but also on environmental factors, such as soil moisture content and texture (Nosratti et al., 2018). Indeed, Zhang et al. (2021) have reported that germination percentage can predict seedling establishment, but the relationship was largely dependent on soil moisture conditions in the field. Therefore, the level of dormancy in seeds and environmental conditions should be taken into consideration when predicting field seedling emergence and survival based on laboratory germination traits.

Implications for ecological restoration

We showed that dry storage significantly increased seedling emergence and survival in the field, suggesting that the use of dry stored seeds in ecological restoration projects may achieve enhanced recovery of vegetation. In addition, dry storage decreased the intraspecific variation of germination traits, but it had little effect on the intraspecific variation in seedling emergence and survival, indicating that it is feasible to use dry storage seeds to evaluate and select suitable provenances for use in ecological restoration. Further, considering that the temperature threshold for germination of fresh and stored seeds are not related to seedling emergence and survival, it is necessary to be very cautious about predicting seedling performance in the field based on seed germination traits in the laboratory, at least for *E. nutans*.

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