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COMPARISON OF APPROPRIATE TROPICAL SEED STORAGE TECHNIQUES FOR GERMPLASM CONSERVATION IN MOUNTAINOUS SUB-TROPICAL CLIMATES WITH RESOURCE CONSTRAINTS

By MARCIA CROFT†, ABRAM BICKSLER‡,§, JAMES MANSON† *and* RICK BURNETTE†

†*Educational Concerns for Hunger Organization (ECHO) Asia Impact Center Seed Bank, 121 M. 8 Tambon Maenawan, Ampur Mae Ai, Chiang Mai, Thailand 50280 and* ‡*International Sustainable Development Studies Institute (ISDSI), 48/1 Superhighway (Chiang Mai-Lamphun Rd.), Muang, Chiang Mai, Thailand 50300*

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SUMMARY

Changes in seed viability over 12 months of low-input storage conditions were monitored on five diverse seed species grown in the tropics: amaranth (*Amaranthus cruentus*), lablab bean (*Lablab purpureus*), moringa (*Moringa oleifera*), pumpkin (*Cucurbita moschata*) and tomato (*Solanum lycopersicum*). Because the costs of maintaining low-temperature storage can be prohibitively expensive in developing countries, this study explored alternatives to low-temperature storage at the Educational Concerns for Hunger Organization (ECHO) Asia Impact Center Seed Bank in Northern Thailand. Specifically, this research compared the effects of vacuum sealing and refrigeration on stored seed viability in both laboratory and field settings. While seed species was an influential factor in determining seed longevity, the relative importance of vacuum sealing and refrigeration differed for the dependent variables of seed moisture content, germination rate, mean time to 50% germination and field emergence. Although the combination of vacuum sealing and refrigeration was most effective at conserving seed quality as measured by each of these variables, the storage of seeds in vacuum-sealed packages at ambient temperatures was more effective than unsealed but refrigerated packets at conserving low moisture content and high germination and field emergence rates across species. This suggests that for resource-constrained seed banks in the tropics, vacuum sealing with or without refrigeration may represent a viable alternative to other expensive and energy-intensive storage techniques.

INTRODUCTION

Conserving the genetic base of food crops around the world requires an in-depth understanding of both environmental and biological variables affecting stored seeds from both temperate and tropical regions. While most research has been focused on temperate species, much of the world's most threatened crop biodiversity exists in the developing countries in the tropics, where resources for optimum storage of germplasm are most limiting (Rao *et al.*, 2006). Determining methods for high-quality germplasm storage under resource-constrained conditions could help conserve these crucial genetic resources. Tropical seeds differ biologically from temperate seeds in many ways, but the environmental factors that affect the rate of deterioration in

§Corresponding author. Email: abram.bicksler@gmail.com

storage remain the same: oxygen concentration, moisture content of the seeds and temperature (Roberts, 1973). An increase in any of these factors will lower the viability of the seeds, and each contributes to cellular degradation in particular ways.

While oxygen is the primary initiator of free radical generation (Bailly, 2004), seed moisture content is the most important factor for determining the rate of deterioration (Rao *et al.*, 2006). At high seed moisture contents (above 14%), oxidative enzymes, such as lypoxygenase, actively generate free radicals that damage cells (Bailly, 2004; McDonald, 1999). Elevated temperatures not only speed up the rate of deteriorative reactions but also increase the ability of air to suspend water, leading to an interaction between temperature and seed moisture content that multiplies the negative effects of these variables on seeds (McDonald, 1999). The International Plant Genetics Resources Institute (IPGRI) advocates drying seeds to $5 \pm 2\%$ moisture content for storage at $-18°C$ (Zeng *et al.*, 1998), although the cost of maintaining a low-temperature and low-moisture storage facility can be prohibitively expensive, especially in tropical developing countries.

To find appropriate ways of conserving this biodiversity, research has been conducted on seed bank storage conditions that can maximize longevity of seeds while minimizing costs (Chiu *et al.*, 2003; Ellis, 1998). Research on seed storage under non-refrigerated conditions suggests that seed moisture may be more important than temperature in determining longevity (Ellis, 1998). Exposure to ambient humidity can be effectively minimized by vacuum sealing, and even a partial vacuum has proven successful in extending seed longevity (Chiu *et al.*, 2003; Yeh *et al.*, 2005). This suggests that in conditions where refrigeration may not be feasible, vacuum sealing could offer a less energy-intensive solution.

Focusing research on local conditions and individual seed banks' needs will provide more context-specific solutions. The purpose of this study was to investigate the most appropriate storage method for the Educational Concerns for Hunger Organization's (ECHO) Asia Impact Center Seed Bank in Northern Thailand as a representative model for other low-input seed banks in the developing world. The goal of this seed bank is to distribute seeds of underutilized crops to development workers throughout Asia, and this research tested the hypothesis that seed viability of five key tropical species is sufficiently conserved under vacuum-sealed conditions to replace refrigeration and minimize costs. Specifically, this study evaluated the effects of ambient humidity and gas removal via vacuum sealing to distinguish these from the effects of reduced temperatures on stored seed viability in both laboratory and field settings. Taking into account the recommendations of international agencies, such as IPGRI, the outcomes of this study will help to prescribe storage conditions for this seed bank and others like it, thereby empowering local actors in the conservation of crop biodiversity.

MATERIALS AND METHODS

Study site and seed materials

The study was conducted at the ECHO Asia Impact Center Seed Bank located near Mae Ai, Chiang Mai province, Thailand (20°1′ N, 99°17′ E). All seed treatments

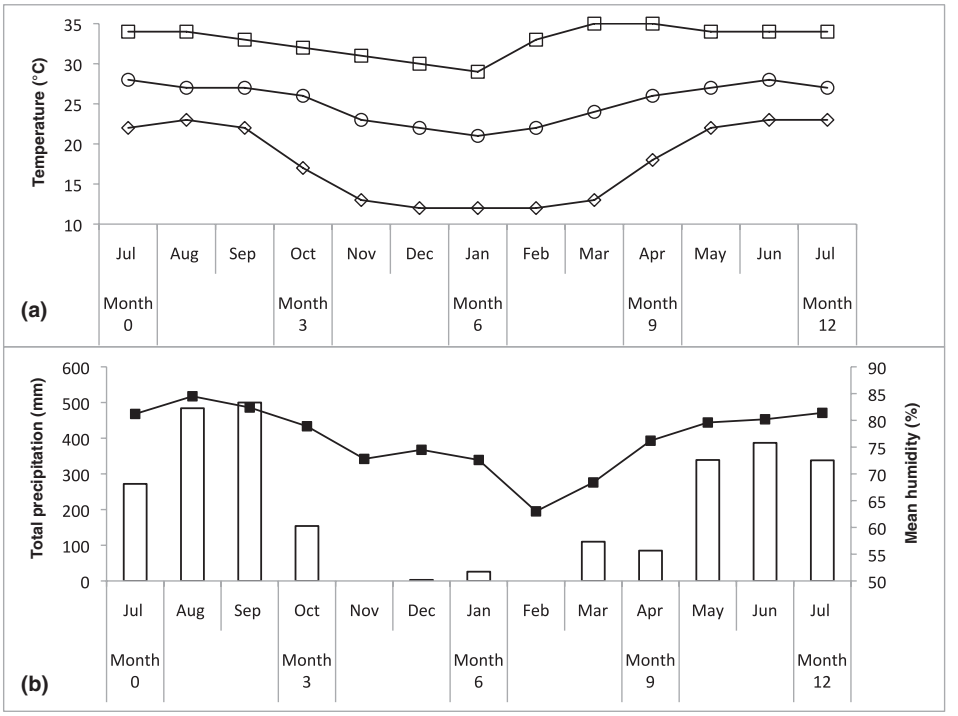

Figure 1. Monthly average values of (a) maximum (squares), mean (circles) and minimum (diamonds) air temperatures (°C), and (b) total mm precipitation (bars) and mean percentage humidity (squares) from July 2010 to July 2011 in Chiang Rai, Thailand (19°54′ N 99°49′ E). Treatment began on July 2010 and measurements were taken every three months.

were stored from 13 July 2010 to 29 July 2011, encompassing a cycle of rainy, dry and hot seasons (Figure 1). Seed storage took place in an insulated room, but temperature, relative humidity (RH) and light varied with ambient conditions.

This research used orthodox seeds from diverse taxa that grow in the tropics, including amaranth (*Amaranthus cruentus*, 'USDA PI 606767'), lablab bean (*Lablab purpureus* 'Chiang Dao'), moringa (*Moringa oleifera* 'Phetchabun'), pumpkin (*Cucurbita moschata* 'Nang kaang kot') and tomato (*Solanum lycopersicum* 'Juliet 1437'), all collected from several local sources. Seeds were sorted for quality: mold- and pest-free seeds were separated visually, while damaged or diseased seeds were discarded.

Experimental design

Three experiments were conducted to determine the effects of seed species and storage method on (1) seed moisture content, (2) seed germination and (3) field emergence. Each experiment was conducted as a factorial in a randomized complete block design with four replications. Fixed factors were seed species (amaranth, lablab, moringa, pumpkin and tomato) and the following four low-input storage methods: vacuum sealing/refrigeration (VR), vacuum-sealing/non-refrigeration (VN), paper

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packets/refrigeration (PR) and paper packets/non-refrigeration (PN). Vacuum sealing treatments were intended to keep seeds at a more constant ambient moisture level, while refrigeration provided constant ambient temperature. An additional fixed factor included storage time (0, 3, 6, 9 and 12 months of storage).

Experimental units consisted of five seeds for the seed moisture content experiment (or 1.90 g for amaranth, owing to the small size of the seeds, which made seed moisture content determination difficult), a petri dish of 20 seeds for the seed germination experiment and a pot of three seeds for the field emergence experiment. Each experimental unit was replicated four times for each experiment at each sampling period. Due to time and cost constraints, the 28 seeds used for the three experiments were sealed together based on seed species and storage treatment outlined above. The four dependent variables measured for each of the respective experiments were seed moisture content (experiment 1), seed germination rate and mean time to 50% germination (experiment 2) and field emergence (experiment 3). Baseline seed moisture content, seed germination rate and mean time to 50% germination were determined for all seed species before sealing treatments had begun (sampling period 0).

Seed drying and storage

In order to attain a standardized moisture level at the onset of the experiment, bulk lots of each seed species were dried for 8 h in a forced-air seed dryer at 33.8 °C ± 2°C and 65 ± 15% RH. All constant moisture treatments were vacuum-sealed to 0.080 MPa (DZ-320A Vacuum Packing Machine, Hongzhan, China) in clear plastic bags, while fluctuating moisture treatments were stored in unsealed paper envelopes. Constant temperature treatments were randomly placed in a refrigerator (Sanyo, Panasonic Management (Thailand) Co., Bangkok, Thailand) and maintained at 5.5 °C ± 2°C and 78 ± 20% RH for the duration of the study. Fluctuating temperature treatments were stored indoors at ambient conditions (Figure 1).

Seed moisture content determination

Moisture content determination took place by combining the four replications of five seeds (1.90 g for amaranth) into two replications with a sample size of 10 seeds for each seed species (Hanson, 1985). Before sealing treatments had begun, baseline moisture content by seed species was determined on 2 July 2010 using four replicates of 45 seeds (7.20 g of amaranth); a larger sample size was used to ensure greater accuracy. Moisture content was measured directly using a low constant temperature oven-drying method and expressed on a fresh weight basis (Sastry *et al.*, 2007). Tomato seeds were excluded from analysis of seed moisture content due to small seed size and inadequate mass for moisture analysis.

Seed germination determination

Germination rate was determined using modified procedures for petri dish germination as prescribed by Rao *et al.* (2006). Four replications of 20 seeds for each

treatment were used at each sampling period and before treatments were applied. Seeds were sterilized in a 10% bleach solution to reduce contamination. Petri dish experimental units were randomly placed within the replications in a germination cabinet maintained at a temperature of 30°C ± 5°C by two 10 W fluorescent light bulbs timed to turn on and off every 90 min. Germinated seeds were recorded and removed every other day for a period of 14 days; germination was defined as the emergence of the seed radicle from the seed coat, and mean time to 50% radicle emergence was also calculated (Hanson, 1985). Tomato seed mean time to 50% germination in month 12 was unable to be included in this analysis due to inadequate degrees of freedom as many of the replications had to be dropped due to 0% germination.

Field emergence determination

Field emergence was determined from four replications of three seeds, planted together in plastic bag pots (8 cm diameter × 15 cm height) filled with equal parts of clean rice husk, composted cow manure and native soil, all obtained from local sources. Each seed was considered a sub-sample, and pots were considered as experimental units. Pots were placed in a partial sun seed nursery as a randomized complete block design with four replications and watered consistently as needed. Field emergence (defined as any plant growth observed above the soil surface) was measured every five days for a period of 30 days to determine field emergence rate (TeKrony and Egli, 1977).

Statistical analysis

All data were analyzed with analysis of variance (ANOVA) and Fisher's Least Significant Difference (LSD) *post hoc* tests using the Mixed Procedure of SAS and PDMIX800 macro (Saxton, 1998) for determining differences between seed species and treatments at each sampling period for each experiment. For all dependent variables, degrees of freedom were adjusted using the Satterthwaite correction (Littell *et al.*, 2002), and normality of the raw data and residuals was evaluated using the UNIVARIATE procedure of SAS. Although ANOVA included interaction effects, we limited our inference space to the main effects of seed type and storage treatment at each sampling period to assist the ECHO Asia Impact Center discern best practices for seed storage treatments and create baseline data for seed species decomposition over time. Sampling date was regressed with all dependent variables for seed type and seed storage treatment using the linear or quadratic function of the REG Procedure of SAS. In addition, relationships between seed moisture content, germination rate, mean time to 50% germination and field emergence were analyzed for correlation using the CORR Procedure of SAS.

RESULTS

Seed moisture content

After an initial increase between baseline measurements and the month 3 sampling period, seed moisture content levels reached a plateau, as modeled by the quadratic

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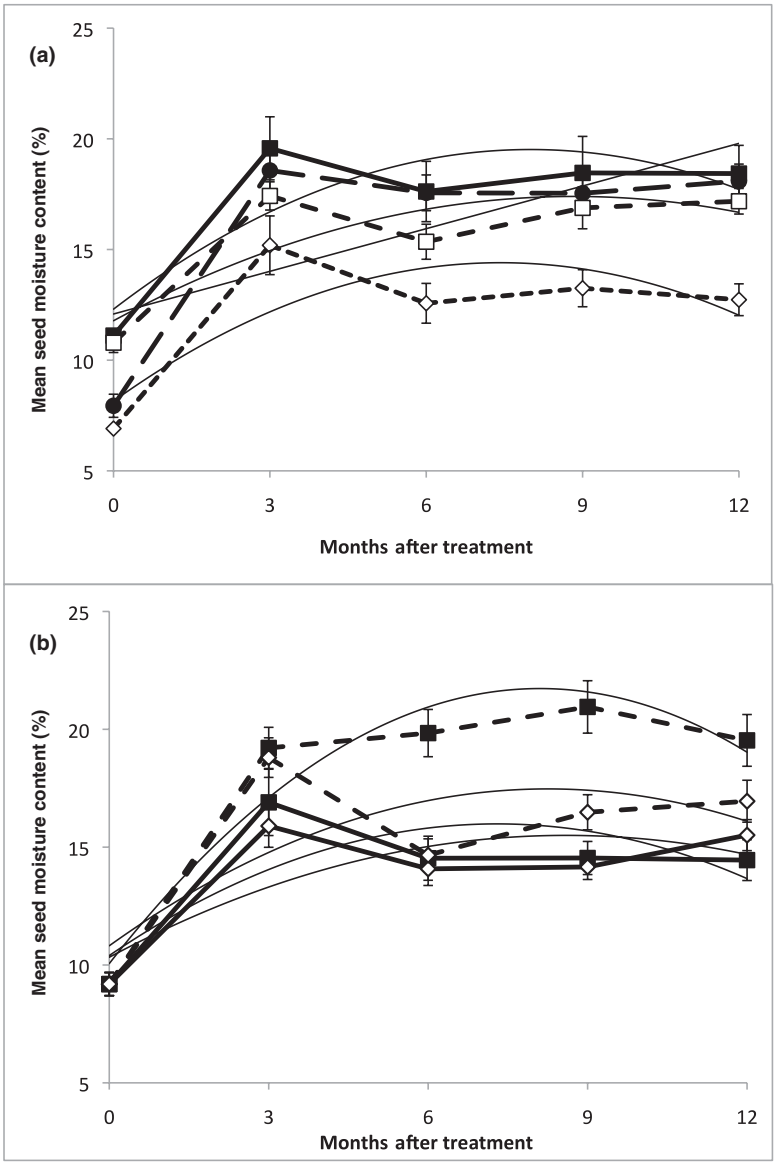

Figure 2. Mean seed moisture content (%) and corresponding trendlines (a) as a function of seed type and months, and (b) as a function of seed storage treatment and months after treatment. (a) Symbols denote lablab (■), pumpkin (●), amaranth (□) and moringa (◊) seeds. (b) Symbols denote VR (■, solid line), VN (◊, solid line), PR (■, dashed line) and PN (◊, dashed line) treatments. Error bars represent ±1 standard error of the mean.

regression equations (Figure 2, Table 2), and moisture content was the only dependent variable where the main effect of sampling period was not significant ($F = 4.5$, $p = 0.09$). The main effect of seed species on moisture content was highly significant over the course of the experiment ($F = 45.4$, $p < 0.0001$); lablab seeds consistently had the highest moisture content, while moringa seeds had significantly lower moisture

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content than every other species at all sampling periods in which there were significant differences (Figure 2a).

During the last three sampling periods, there were also significant differences between storage treatments (Table 1, Figure 2b). The PR treatment had significantly greater seed moisture content than all other treatments, with a cumulative average of 19.8% moisture, almost 5% higher than vacuum-sealed treatments. The two vacuum-sealed treatments consistently maintained the lowest seed moisture content levels over the 12-month period regardless of refrigeration, but were not significantly different from each other at any sampling period.

Germination rate

Seed germination rates declined over the 12-month period across species and treatments from the initial baseline average of 76.3% to 45.8% by month 12, although some seeds and treatments experienced a slight increase at month 3 (Figure 3, Table 2). The main effect of seed species also significantly affected germination rate (Table 1) and seed species fell into three statistically different groups across the experiment ($F = 42.9$, $p < 0.0001$): lablab seeds exhibited the highest germination rates, amaranth and pumpkin seeds exhibited moderate rates and moringa and tomato seeds exhibited the lowest germination rates (Figure 3a).

The main effect of seed storage treatment on germination rate was also highly significant ($F = 49.1$, $p < 0.0001$) across sampling periods. Storage treatments differed significantly from each other across the experiment (Figure 3b), falling approximately 10% in germination between each treatment from VR to VN, PR and PN respectively. Differences between seed storage treatments were magnified over the 12-month period to almost 20% by month 12, from the highest (VR, at 75.8%) to the lowest (PN, at 8.6%) (Figure 3b) as modeled by quadratic regression equations for each treatment (Table 2). There was no significant correlation between moisture content and germination rate ($r = 0.06$, $p = 0.65$).

Mean time to 50% germination

The main effect of seed species on mean time to 50% germination was highly significant ($F = 233.2$, $p < 0.0001$) across all sampling periods (Figure 4a, Table 2), while the effect of storage treatment was comparatively slight ($F = 19.6$, $p < 0.0001$) (Figure 4b, Table 2). There was a wide range of baseline values for mean time to 50% germination across seed species (Figure 4a), and mean times to 50% germination generally stayed within the range of these baseline measurements. Each seed species was significantly different from all others across the experiment, from moringa with the longest time (9.4 days), decreasing to tomato, pumpkin, amaranth and lablab seeds (2.9 days) (Figure 4a). There was a significant negative correlation between germination rate and mean time to 50% germination ($r = -0.71$, $p < 0.0001$).

The main effect of seed storage treatment also had a significant effect on mean time to 50% germination at each sampling point, although the F-values were small relative to the effect of seed species (Table 1). All treatments consistently increased or decreased

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Table 1. Summary of F-values from Analysis of Variance at each of the five sampling periods for the dependent variables of moisture content, germination rate, mean time to 50% germination and field emergence. Extreme outliers were excluded, and normality of raw data and residuals were evaluated using the UNIVARIATE procedure of SAS. Significance levels are denoted by asterisks.

	Moisture content			Germination rate			Mean time to 50% germination			Field emergence		
Month	Seed species	Treatment	Seed species × treatment	Seed species	Treatment	Seed species × treatment	Seed species	Treatment	Seed species × treatment	Seed species	Treatment	Seed species × treatment
0	53.97***	N/A	N/A	6.78**	N/A	N/A	13.14**	N/A	N/A	N/A	N/A	N/A
3	3.08	2.17	0.82	12.46***	12.43***	2.85	137.49***	8.46***	4.86***	2.88*	4.30**	2.34*
6	20.21***	24.11***	2.20	13.50***	4.80**	1.95*	127.92***	3.93*	1.91	9.11***	6.62**	2.28*
9	4.96***	83.55***	4.19**	21.32***	11.39***	3.40**	49.42***	4.31**	0.72	2.81*	3.28*	0.84
12	40.09***	27.88***	2.39	23.46***	63.51***	5.59***	33.66***	5.23**	1.79	2.86*	27.94***	1.42

*$p < 0.05$ using Fisher's Protected LSD with the PDMIX800 macro in the MIXED Procedure of SAS.
**$p < 0.01$ using Fisher's Protected LSD with the PDMIX800 macro in the MIXED Procedure of SAS.
***$p < 0.0001$ using Fisher's Protected LSD with the PDMIX800 macro in the MIXED Procedure of SAS.

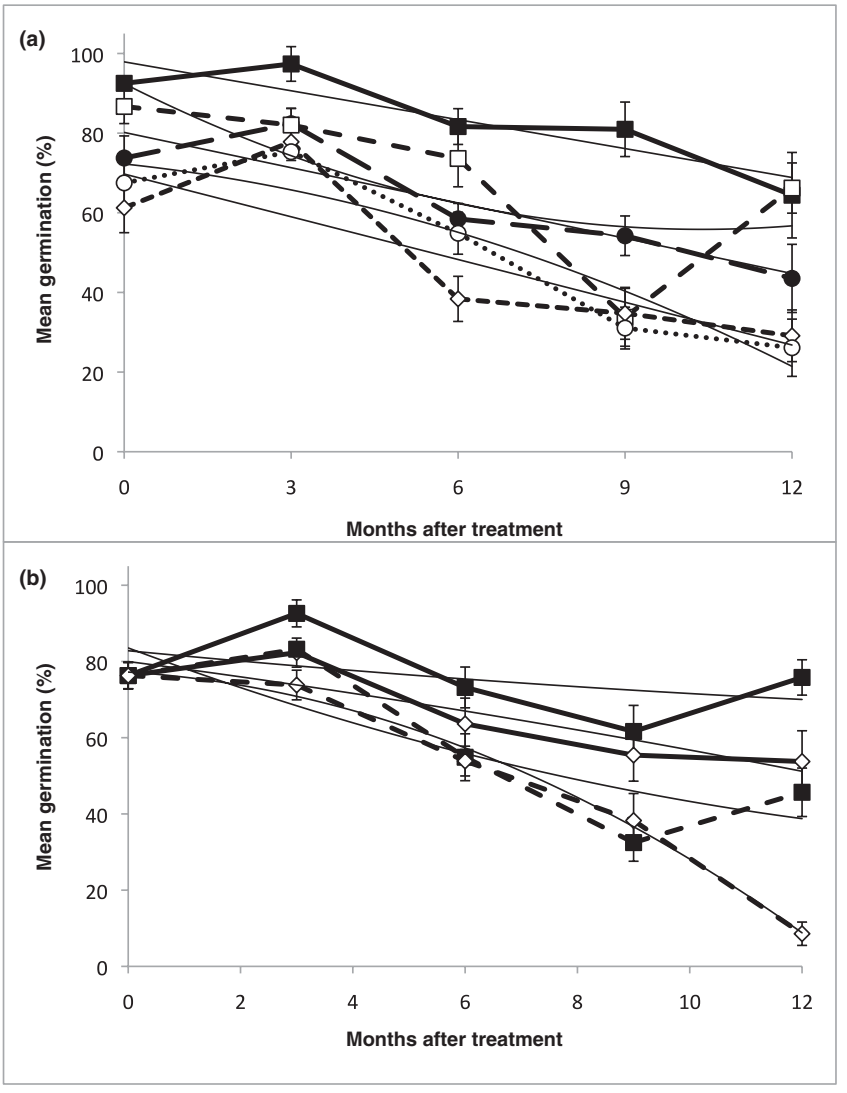

Figure 3. Mean seed germination (%) and corresponding trendlines (a) as a function of seed type and months after treatment, and (b) as a function of seed storage treatment and months after treatment. (a) Symbols denote lablab (■), pumpkin (●), amaranth (□), moringa (◇) and tomato (○) seeds. (b) Symbols denote VR (■, solid line), VN (◇, solid line), PR (■, dashed line) and PN (◇, dashed line) treatments. Error bars represent ±1 standard error of the mean.

together at each sampling period, although not to the same degree (Figure 4b, Table 2). By month 12, the two non-refrigerated treatments had significantly greater mean time to 50% germination than the VR treatment (Figure 4b), and although the PR treatment had lower mean time to 50% germination than the VN treatment at each sampling period, their averages were not significantly different.

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Table 2. Regression equations and coefficients of regressions for the effects of seed species and seed storage treatment across sampling periods on the dependent variables of seed moisture content, germination rate and mean time to 50% germination.

Seed moisture content							
Seed species	Equation	R^2	Best fit	Treatment	Equation	R^2	Best fit
Amaranth	$-0.10x^2 + 1.63x + 12.04$	0.40	Quadratic	PN	$-0.11x^2 + 1.84x + 10.05$	0.61	Quadratic
Lablab	$-0.06x^2 + 1.05x + 13.85$	0.45	Quadratic	PR	$-0.17x^2 + 2.78x + 9.79$	0.89	Quadratic
Moringa	$-0.10x^2 + 1.40x + 9.13$	0.15	Quadratic	VN	$-0.08x^2 + 1.36x + 9.79$	0.67	Quadratic
Pumpkin	$0.44x + 13.24$	0.22	Linear	VR	$0.11x^2 + 1.66x + 9.83$	0.65	Quadratic
Germination rate							
Seed species	Equation	R^2	Best fit	Treatment	Equation	R^2	Best fit
Amaranth	$0.61x^2 - 11.43x + 107.86$	0.20	Quadratic	PN	$-0.43x^2 - 0.23x + 73.68$	0.60	Quadratic
Lablab	$-3.09x + 103.08$	0.17	Linear	PR	$0.14x^2 - 5.00x + 79.32$	0.30	Quadratic
Moringa	$-4.33x + 75.03$	0.30	Linear	VN	$-0.01x^2 - 0.66x + 80.01$	0.14	Quadratic
Pumpkin	$-3.51x + 85.16$	0.26	Linear	VR	$-0.01x^2 - 0.66x + 80.01$	0.03	Quadratic
Tomato	$-0.06x^2 - 4.40x + 83.31$	0.43	Quadratic				
Mean time to 50% germination							
Seed species	Equation	R^2	Best fit	Treatment	Equation	R^2	Best fit
Amaranth	$0.26x + 1.68$	0.14	Linear	PN	$-0.01x^2 + 0.33x + 4.78$	0.10	Quadratic
Lablab	$0.01x^2 + 0.04x + 2.12$	0.30	Quadratic	PR	$0.14x + 4.57$	0.03	Linear
Moringa	$0.06x^2 + 0.99x + 5.90$	0.26	Quadratic	VN	$0.0004x^2 + 0.20x + 4.74$	0.04	Quadratic
Pumpkin	$-0.01x^2 + 0.32x + 3.23$	0.21	Quadratic	VR	$0.01x^2 + 0.0001x + 4.70$	0.01	Quadratic
Tomato	$0.13x^2 - 0.75x + 6.95$	0.57	Quadratic				

Field emergence

The dependent variable of field emergence was strongly correlated with germination rate ($r = 0.57$, $p < 0.0001$) and less strongly with mean time to 50% germination ($r = -0.22$, $p = 0.06$). The main effect of seed species on field emergence, while significant, was relatively small compared to its effect on other dependent variables at each sampling period (Table 1). Similar to germination rate, lablab seeds had significantly higher field emergence (averaging 78.2%) than all other species across sampling periods, but there were no significant differences between other seed species (Figure 5a).

Although baseline field emergence was not assessed before treatments began, there were significant differences in field emergence between treatments at each of the subsequent sampling periods (Table 1). The VR and PN treatments had average field emergence rates of 85.0% and 8.3% at 12 months respectively, which were both almost identical to their respective germination rates (Figures 3b and 5b). Refrigerated treatments had significantly higher overall field emergence rates than non-refrigerated treatments, and the same was true for vacuum-sealed treatments over unsealed treatments (Figure 5b). The overall field emergence data mirror germination rates in the rank order of treatments, although the differences between VN and PR treatments were not as significant for the dependent variable of field emergence as they were for germination rate (Figures 3b and 5b).

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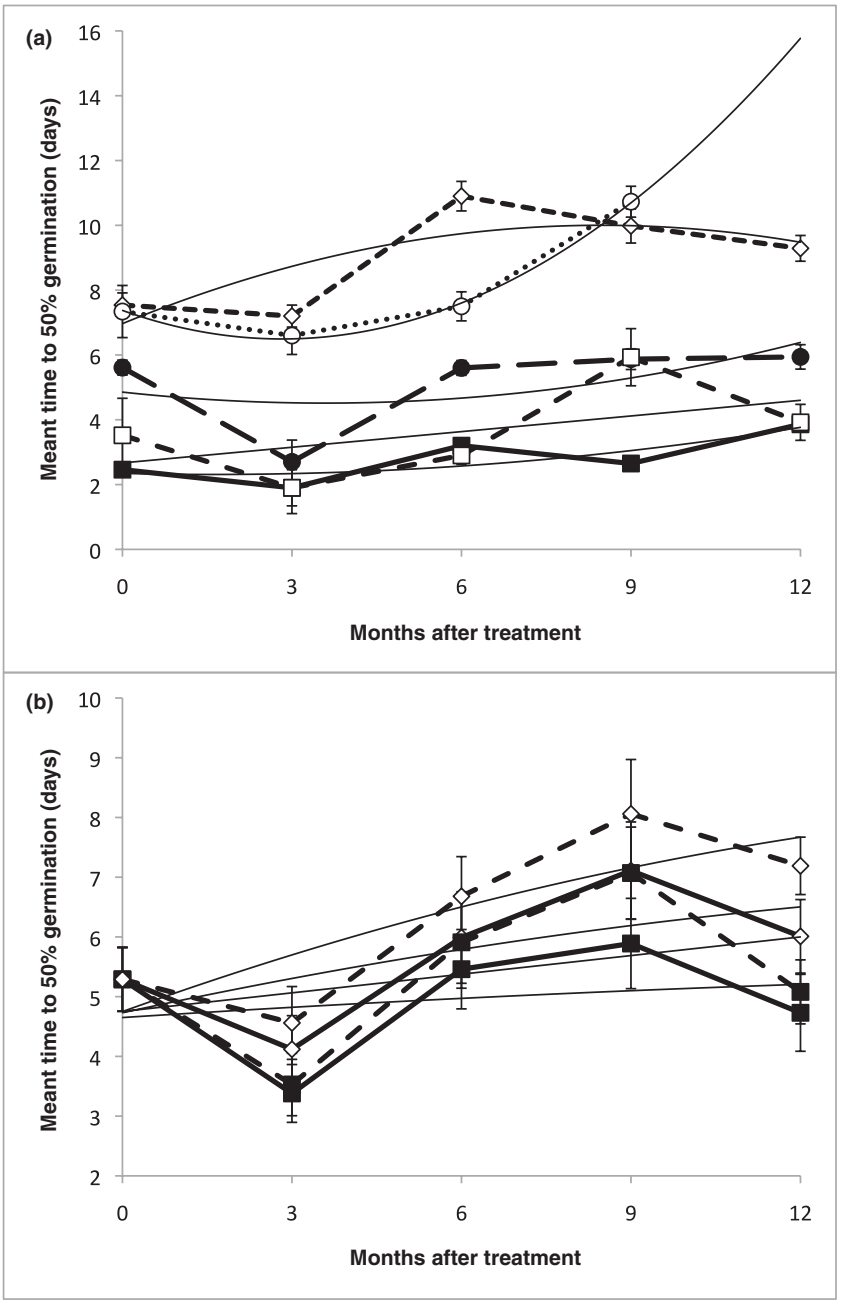

Figure 4. Mean time to 50% germination (days) and corresponding trendlines (a) as a function of seed type and months after treatment, and (b) as a function of seed storage treatment and months after treatment. (a) Symbols denote lablab (■), pumpkin (●), amaranth (□), moringa (◊) and tomato (○) seeds. (b) Symbols denote VR (■, solid line), VN (◊, solid line), PR (■, dashed line) and PN (◊, dashed line) treatments. Error bars represent ±1 standard error of the mean.

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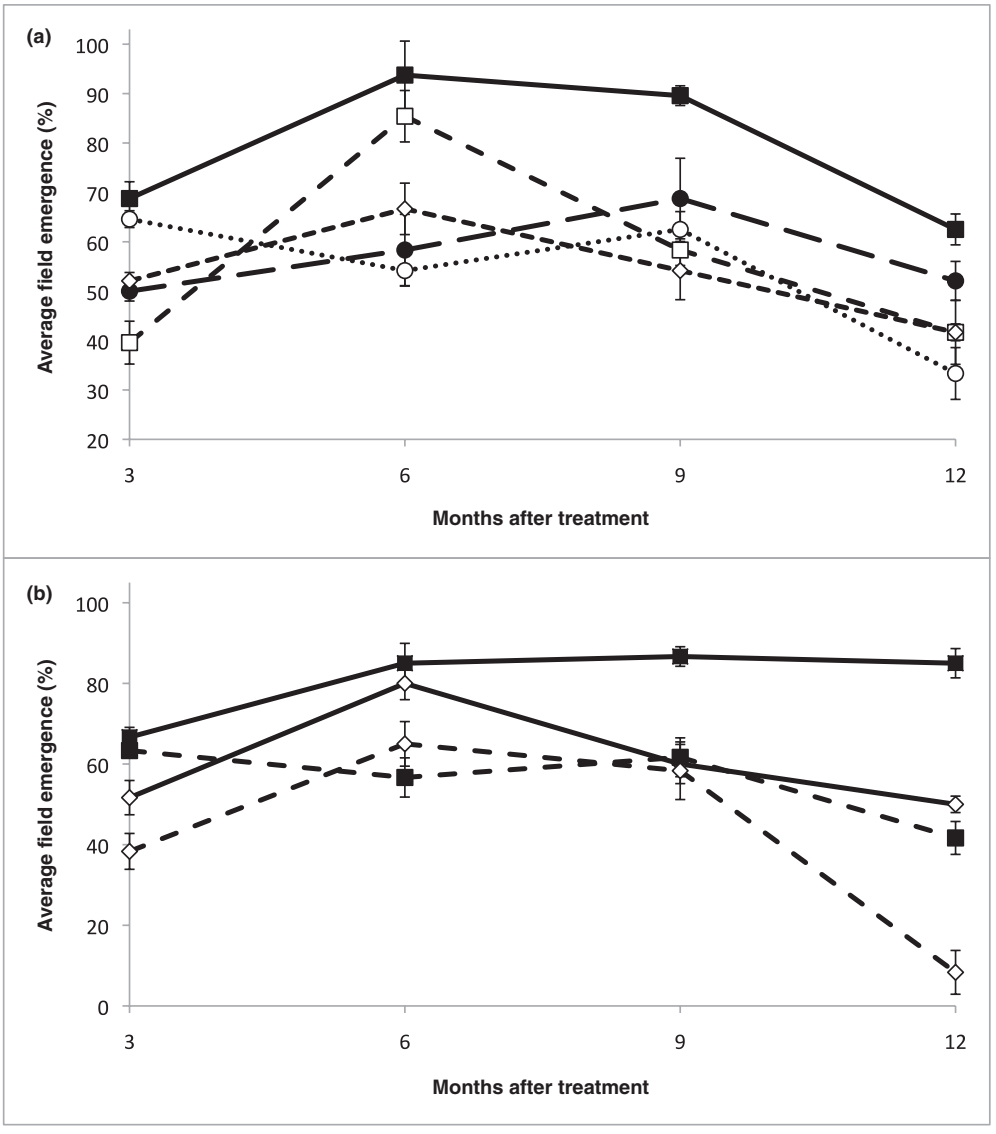

Figure 5. Mean field emergence (%) (a) as a function of seed type and months after treatment, and (b) as a function of seed storage treatment and months after treatment. (a) Symbols denote lablab (■), pumpkin (●), amaranth (□), moringa (◊) and tomato (○) seeds. (b) Symbols denote VR (■, solid line), VN (◊, solid line), PR (■, dashed line) and PN (◊, dashed line) treatments. Error bars represent ±1 standard error of the mean.

DISCUSSION

Seed moisture content

Seed moisture content remained stable throughout the experiment after an initial spike in month 3, which may be due to the 8 h of drying at the onset of the experiment used to standardize moisture levels before storage treatments began. Moisture content

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varied significantly by seed species but not by storage duration, indicating that inherent differences between seeds' moisture contents remained strong over time, as suggested by Egli and TeKrony (1997). This might mean that some seeds (such as moringa) require fewer resources to conserve low seed moisture content even over long periods of time; taking these qualities into consideration can help seed banks allocate resources more effectively.

The difference in seed moisture content between sealed and unsealed treatments was dramatic, and most evident in the PR treatment (Figure 2b). This high moisture level may have been caused by the extremely high humidity levels in the refrigerator, sometimes as high as 98%. Outside the refrigerated environment, the PN treatment had significantly lower moisture content, suggesting that the lack of sealing alone was not responsible for differences in the PR treatment. The VN and VR treatments were never significantly different from each other, suggesting that vacuum sealing with or without refrigeration consistently minimizes moisture content and moderates fluctuation. Despite research (Ellis, 1998; McDonald, 1999) showing that high levels of ambient humidity place seeds at risk from the deteriorative reactions, our data showed no significant correlation between moisture content and germination rate ($r = 0.06$, $p = 0.65$). Conserving low moisture content may be important for preventing seed degradation, but it is possible that these effects did not become apparent after only one year of storage and a longer study may be necessary to more adequately assess degradation. The main effects of both seed species and storage treatment significantly affected germination rate and these may have also outweighed the influence of seed moisture content.

Seed germination rate

Significant differences between seed species in germination rates demonstrate important biological differences influencing deterioration in storage; significantly higher germination rates of lablab seeds suggest that fewer measures may be necessary to conserve germination for this species (Figure 3a). The initial quality of these seeds may have been higher than others, however, as their baseline germination rate was significantly higher than all other species except amaranth (Figure 3a). Sudden changes in atmospheric conditions can trigger or halt the germination of certain species (Bradbeer, 1988), which may explain the slight increase in germination of some seed species and treatments at month 3 as precipitation and humidity dropped dramatically during this month (Figure 1).

Seed germination rates declined across all treatments over the experimental period, although refrigeration or lack thereof seemed to determine the trajectory of decline, while sealing treatments affected the rate of decline. Vacuum sealing may have helped lower the rate of viability loss by minimizing oxygen pressure and exposure to free radicals (Bailly, 2004; Yeh *et al.*, 2005), while the temperature of storage may have determined the overall trajectory of decline by impacting the rate of cellular decay (McDonald, 1999). Both refrigeration and vacuum sealing helped conserve seed germination rate, but between the two, vacuum sealing alone was more effective than refrigeration. Overall, trends suggest that if conserving seed germination is of

primary importance, vacuum sealing and refrigerating seeds is ideal, but vacuum sealing alone may be sufficient to lower the rate of viability loss.

Mean time to 50% germination

Accumulating cellular decay eventually causes seed death, but an increased mean time to 50% germination may be an early sign of declining seed quality. A strong correlation between mean time to 50% germination and germination rate ($r = -0.72$, $p < 0.0001$) suggests that this may be a good predictor of other factors affecting seed quality. The mean time to 50% germination also reflects important biological differences between species, as the main effect of seed species had a greater effect on this variable than any other measured variable (Table 1). Each species generally stayed within a narrow range (Figure 4a), suggesting that seed species have very different inherent patterns of germination that do not alter extensively during storage.

Treatments followed a consistent pattern in mean time to 50% germination, although differences were not always significant. Refrigerated treatments had the lowest mean time to 50% germination rates, suggesting that seeds' ability to germinate quickly is more effectively conserved by refrigeration than vacuum sealing. The data suggest that while both vacuum sealing and refrigeration are important for preserving seeds' germination rate and mean time to 50% germination, refrigeration plays a greater role than vacuum sealing in the latter but not the former. While temperature is critical for slowing the rates of cellular decay in seeds (McDonald, 1999), this decay may begin to negatively impact rate of germination before overall capacity to germinate is compromised. The lack of significant difference between VN and PR treatments also indicates that the effect of refrigeration on mean time to 50% germination is not as great as the effect of vacuum sealing on germination rate. Their relative importance may vary for each dependent variable (Roberts, 1973), and decisions regarding optimal storage conditions will have to be made by prioritizing aspects of seed quality.

Field emergence

There was more inherent variability in field emergence than germination rate determination, but as TeKrony and Egli (1977) have shown, standard seed germination tests may have a low prediction accuracy for field performance, especially under adverse field conditions. Unlike germination rate, field emergence rates did not consistently segregate by species and might have been more vulnerable to the climate over sampling periods, responding better during their regular planting seasons. A strong correlation between germination rate and field emergence shows that under these conditions germination rate can still act as a good predictor of field emergence.

Although differences between VN and PR treatments were not significant, both germination rate and field emergence were better conserved through vacuum sealing than refrigeration. Both these variables point to the same trend: vacuum sealing and refrigeration together best conserve seed germination but vacuum sealing alone may be more effective than just refrigeration.

CONCLUSION

Appropriate seed storage methods for each gene bank will invariably depend on the goals and resources available in each situation. Maintaining storage conditions that keep oxygen pressure, moisture content and temperature at a minimum can be an expensive but effective way of preserving germplasm at the highest quality (Chiu *et al.*, 2003; Rao *et al.*, 2006; Roberts, 1973). If resources are a constraining factor and only vacuum sealing or refrigeration is an option, then the aspect of seed quality that is most important will be the determining factor. Considerations must also include the type of seeds that are in storage, as the rate of degradation is often species-specific.

For each dependent variable measured, the vacuum-sealed and refrigerated treatment across all seeds was the best performing treatment, although the relative benefits of vacuum sealing and refrigeration alone differed for each variable. For conserving a fast rate of mean time to 50% germination, refrigeration rather than vacuum sealing was most effective. For every other dependent variable (seed moisture content, germination rate and field emergence), vacuum sealing had a greater positive effect on conserving seed viability, suggesting that vacuum sealing may be the most appropriate way to conserve seed quality at low cost.

Differences in seed species may also be important considerations for optimal storage practices. While some seeds were particularly prone to rapid degradation in storage, others maintained high stored seed quality even when kept at ambient conditions. For example, lablab seeds maintained a higher stored seed quality across all treatments than other seed species and may not require as many resources for storage. It may be more important to spend resources maintaining the quality of seeds most at risk, such as moringa and tomato seeds. Biological differences between seeds had highly significant effects that were as important or more important than storage treatment on most dependent variables. Matching seeds with appropriate storage requirements will most efficiently allocate resources, but this will require a thorough knowledge of each species. Developing a more complete understanding of seed species and storage conditions will allow more widespread conservation of crop germplasm, even under resource-constrained conditions.

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