Enhancing seed conservation in rural communities of Guatemala by implementing the dry chain concept

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Abstract
Seed conservation in rural communities of low- and middle-income countries located in tropical areas is particularly problematic, due to high relative humidity that promotes insect and fungal infestations and leads to rapid losses in seed viability. Seed conservation in those areas is affected by unreliable power supplies that do not allow the use of dehumidifying and refrigeration systems recommended for the long-term storage of seeds. We tested the dry chain, i.e., initial seed drying with a reusable desiccant in the form of zeolite beads followed by seed conservation in hermetic containers, in rural communities of Guatemala (Huehuetenango Department). In this region, a network of community seed reserves (CSRs) has been established to provide a safety backup for seed and to conserve local agrobiodiversity. Using a local maize variety in three communities, we compared the dry chain with the seed conservation methodology employed in the CSRs (i.e., undried seeds in hermetic flasks) as well as with seed conservation in open storage, both in the local CSR and in a farmer’s granary. Seed conserved using the dry chain treatment maintained very high seed viability (> 80%) throughout the whole experiment (6 months) and reduced fungal and insect infestations (< 3%). In the other treatments, the viability declined significantly to an average of 52% non-viable and 19% infested seeds after 6 months of storage. The dry chain was demonstrated to be an excellent solution for enhancing seed conservation in biodiversity hotspots of tropical areas as well as for improving seed security for farmers.

Keywords Seed longevity · Dry chain · Community seed banks · Seed drying · Post-harvest · Seed security · Agrobiodiversity conservation

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Introduction

One of the most effective strategies to ensure the conservation and availability of plant genetic resources (PGR) is through seed storage (Davies and Allender 2017). Seed conservation shows several advantages as a medium/long-term conservation strategy for plant biodiversity: seed material is relatively easy to collect, can be stored in small spaces, can provide a representative sample of the genetic diversity, and, under the right conditions, remains viable for long periods. Furthermore, seed collections are relatively low maintenance and are economically viable (Li and Pritchard 2009; Riviere and Müller 2018).

Community seed banks, also known as community seed reserves (CSRs), are small-scale local organizations that conserve seeds of landraces and wild useful plants on a medium-term basis (2–10 years) and serve the needs of local communities. CSRs, especially when organized in networks, can have important roles in improving seed security (i.e., “the ready access by rural households to adequate quantities of quality seed of crop varieties, adapted to their agro-ecological conditions and socioeconomic needs”); FAO 2020) and in the conservation of plant agrobiodiversity, defined as: “the diversity of crop species used in different agro-ecosystems as well as the genetic diversity within and among crop and crop wild relatives accessions” (Last et al. 2014; Vernooy et al. 2017). Community seed reserves have been established in various countries of the world over the last 30 years (Vernooy et al. 2015).

In the Sierra de los Cuchumatanes mountain range in the department of Huehuetenango, Guatemala, a network of 18 CSRs has been developed since 2008, supported by several development agencies (Fuentes 2018). In this CSR network, located in a biodiversity hotspot and a center of crop origin and diversity of Mesoamerica (Vavilov 1926; Myers et al. 2000), almost 900 different seed accessions of local plant genetic resources (including crop landraces, crop wild relatives and wild medicinal plants) are conserved (S. Alonzo, personal communication 2020). Additionally, several landraces of maize particularly adapted to local conditions are stored in those CSRs and can be distributed to any local farmers to reactivate agricultural production in the case of extreme climatic events (Fuentes 2018). These CSRs also offer a critically important free service to more than 800 local farmers by conserving, as a safety duplicate, a sample of their seeds (1–2 kilos in the case of maize) for the short-term (generally one year), allowing them to retrieve those seeds for planting and protecting against complete loss of their germplasm. The CSR network of the Sierra de los Cuchumatanes strengthens the seed and food security of more than 5000 people in the region and has particularly enhanced the conservation of local landraces (Galluzzi and Lapeña 2015).

Seed conservation is particularly difficult in humid tropical areas, since high environmental temperatures and high relative humidity detrimentally affect seed longevity in storage (Roberts 1973; Ellis and Roberts 1980; Dickie et al. 1990), as well as increase the proliferation of fungi and insects, leading to rapid losses in seed viability (Welty et al. 1987; Pittendrigh et al. 2003). In particular, high seed moisture content is the major factor contributing to seed ageing and post-harvest losses, making it one of the greatest threats for seed and food security in rural communities of the Guatemalan highlands, as well as in many other tropical areas (Afzal et al. 2017; Mendoza et al. 2016). In the CSRs of the study area, the high moisture content of the seed samples is recognized by the local technicians as the greatest threat to seed conservation. Seeds are conserved in hermetic flasks, and no specific initial seed-drying protocol is consistently followed prior to storage; indeed, the seeds that are donated by the farmers are stored in the flasks as received, usually without
any additional treatment. The elevated seed moisture content, due to incomplete drying prior to storage, also leads to major post-harvest losses of seed viability in farmers’ stores in the study area. The farmers of the Guatemalan highlands usually dry seeds on the plant before harvesting and/or dry their seeds in *tapancos* (farmhouse attics) and/or by sun-drying, although this is not always possible due to the rainy conditions around harvesting time (Mendoza et al. 2017). All these drying methods have significant disadvantages: drying in open spaces cannot decrease seed moisture content below a certain level due to the very high environmental relative humidity, and sun-drying can decrease the long-term seed viability (Babiker et al. 2010). Seeds are usually stored by farmers in porous containers in their houses, allowing dry seeds to gain moisture and equilibrate to the high ambient humidity (Mendoza et al. 2016, 2017).

Bradford et al. (2018) proposed that the “dry chain”, i.e., the initial dehydration of seeds to levels preventing fungal growth (RH < 65%) followed by storage in moisture-proof containers, could be an optimal solution for medium-term seed storage in tropical areas, preventing rapid seed ageing as well as fungal and insect infestations. This could be particularly important in low- and middle-income countries where the equilibrium drying in drying rooms followed by cold storage (FAO 2014), typically applied to long-term seed conservation, is not feasible, particularly where power supplies are unreliable. A very effective desiccant material for drying to low equilibrium relative humidity (ERH) is zeolite clay, which can form a microcrystalline pore structure that specifically and tightly binds water (Hay et al. 2012; Hay and Timple 2013). When beads made from zeolite are enclosed in a moisture-proof container with the seeds to be dried, they absorb water from the air and quickly lower the RH within the container. Consequently, without the need for heat, water evaporates from the seeds and is bound to the beads. One commercial version of zeolite drying beads (Drying Beads™) can absorb up to 25% of their dry weight in water, and when saturated, can be fully reactivated for reuse by heating. Silica gel is currently the most frequently used desiccant for seed drying, but zeolite drying beads have a higher water-absorption efficiency at low relative humidity and maintain full efficiency and capacity indefinitely after reactivation (Kunusoth et al. 2012). Seed drying with drying beads followed by conservation in hermetic, moisture-proof containers is being used as the preferred seed conservation method by seed banks and seed companies in several low- and middle-income countries, mainly in Asia (Dadlani et al. 2016; Bradford et al. 2018).

While published studies have demonstrated the effectiveness of zeolite drying beads in seed drying (Hay et al. 2012; Hay and Timple 2013; Kunusoth et al. 2012; Bakhtavar et al. 2019), less evidence is available concerning their applicability under conditions relevant to CSRs (Dadlani et al. 2016). The aims of the current paper are to: (1) assess the effectiveness of the current methodology of seed conservation that is being employed in the CSRs in the Guatemalan highlands; and (2) test the implementation of the dry chain approach in the same region, in particular the use of drying beads to dry seed material coupled with seed conservation in hermetic containers. The trials, comprising three seed storage treatments (i.e. open storage, hermetic storage of untreated seeds, and hermetic storage of seeds dried with zeolite beads), were conducted in three Guatemalan communities, not only in CSRs, but also in farmers’ granaries, to evaluate the effectiveness of the dry chain under both types of seed storage conditions.
Materials and methods

Seed sample preparation

The experiment was performed utilizing a maize accession that resulted from a participatory breeding program in Quilinco (Huehuetenango Department, Guatemala). The seeds were dried on the cob on intact, standing plants in the field and harvested between 10 and 15 January 2019. After manual shelling, the seeds (108 kg) were stored in porous mesh plastic sacks in the community seed reserve of Quilinco until the beginning of the experiment in mid-May 2019.

To test the effectiveness of different seed conservation methods, seeds were stored using three different post-harvest treatments: (1) untreated seeds in mesh bags (code: “Mesh bag” in the graphs); (2) untreated seed material in hermetic flasks (the conservation strategy currently employed by the CSRs; code: “Herm.Flask”); (3) seed material dried for eight days with zeolite Drying Beads™ (Drying Beads 2014; Bradford et al. 2018), and then conserved in hermetic flasks (code: “Beads + Herm.Flask”). The hermetic flasks used in the experiments are plastic jars with sealable lids of the same model as those currently used in the CSRs in the study area (MICROplast. s. a., Guatemala). All the hermetic flasks employed in the experiment were modified by substituting the original opaque plastic lid with a transparent one. A reusable card with a strip of relative humidity (RH) indicator paper embedded in it (Dry Card 2018; EarthEmpower; Bradford et al. 2016; Thompson et al. 2017) was taped to the inside of each clear plastic lid to monitor the relative humidity within the hermetic flask. An RH indicator card was also placed inside each mesh bag for the same purpose. The seed samples were prepared in the headquarters of ASOCUCH (Asociación de Organizaciones de los Cuchumatanes), a local NGO in Chiantla (Huehuetenango Department, Guatemala) that provides technical support to the CSRs. Initial seed moisture contents and subsequent moisture values were obtained using a portable moisture meter (Draminski Twist Grain).

For the two treatments involving undried seeds, 2 kg of untreated maize seeds were transferred into each of 18 mesh bags (treatment: Mesh bag) and into each of 18 hermetic containers (treatment: Herm.Flask) on 22 May 2019.

The drying treatments started on 14 May 2019, when 36 kg of maize of the aforementioned accession were dried for eight days (until 22 May 2019) using drying beads. The seeds to be dried were put in mesh bags (two kilos per bag) and divided into two hermetic plastic boxes (52 cm × 36.5 cm × 27.5 cm DryBoxes™, Dry Chain America). We dried 18 kilos of seed in each box divided into nine mesh bags. The bags of seed in the boxes were interspersed with mesh bags filled with drying beads (11.8 kg of beads per box, placed in 11 mesh bags of 1 kilo and one of 800 g). The mesh bags containing the seed were flattened out, in order to maximize the surface area to favor water exchange. Each layer of seed bags was inserted between two layers of bead bags (above and below). The amount of drying beads to be employed in the drying experiment was determined using the “Drying Beads Calculator”, a calculation tool for the amount of Drying Beads™ needed to dry a certain quantity of crop seeds (Drying Beads 2014). After eight days of drying at room temperature, the moisture content (MC) of four seed samples was measured with the abovementioned moisture meter for each of the two hermetic boxes and the contents of each mesh bag holding the dried seeds were transferred to a single flask (treatment: Beads + Herm.Flask).
Communities involved

After the preparation of the seed samples in Chiantla, they were delivered to the three communities involved in the project, Climentoro (latitude: 15.3869, longitude: -91.4549), Quilinco (lat.: 15.3883, long: -91.351) and San Francisco las Flores (hereafter abbreviated as San Francisco; lat: 15.5796, long: -91.3689), each of which maintains a CSR (Fig. 1). The three communities involved in this experiment are located in the Sierra de los Cuchumatanes mountain range which extends through western Guatemala. This area is characterized by undulating topography with many small valleys and high-plateau formations, with altitudes ranging from 1500 to 3000 m.a.s.l. This mountain range is particularly rich in agrobiodiversity, especially in terms of maize landraces (Porcuna-Ferrer et al. 2020). Most of the precipitation in the study area occurs from May to October, and the mean annual precipitation can exceed 1000 mm. Temperatures vary significantly across altitudes, with the mean maximum and minimum temperatures at high altitude sites (~ 2500 m.a.s.l.) of 19 °C and 7 °C, and 24 °C and 10 °C at lower altitude sites (~ 1900 m.a.s.l.; Caffrey et al. 2011).

The three communities were chosen to maximize the altitudinal range (approx. 1000 m in total, more than 450 m between communities) and temperature regimes experienced by the seeds stored in CSRs throughout the network. Prior to the beginning of the experiment, in April 2019, the relative humidity inside ten randomly selected flasks was measured using RH indicator cards in each of the three CSRs involved, with the RH always being > 75%, which corresponds to an estimated moisture content of > 15.2% in maize seeds (Bradford et al. 2016). In all three communities the seed treatment sets were stored in two sites: a CSR (Fig. 2a) and a farmer’s seed storage area (Fig. 2b). In each site, a seed treatment set of the following nine containers was stored: three mesh bags containing untreated seed, three hermetic flasks containing untreated seed, and three hermetic flasks containing seed dried with beads (Fig. 2c). In close proximity to the sets of containers in each community and site within the community, a datalogger (DataLogger, Centor Thai) was placed to record, at hourly intervals, ambient temperature and relative humidity for the entire duration of the experiment.

Seed viability tests

Seed viability tests were performed at the initiation of the experiment (mid-May) when the containers were delivered to the communities, as well as after 3 and 6 months of storage (mid-August and mid-November 2019, respectively). Seed viability was assessed by seedling emergence tests conducted in a sand bench built in each of the communities close to the CSR, and by germination tests performed on the same dates in an incubator of the Instituto de Ciencia y Tecnología Agrícolas (ICTA) in Villa Nueva, the national seed bank of Guatemala.

Seed quality, seedling emergence and seedling growth

In each community, nearby the CSR, a sand bench (270 cm × 140 cm; 30 cm depth) was built to perform the germination experiments. The sand bench was filled with fine river sand that was previously sifted and then left to sun-dry for 1 week. Each sand bench was covered by chicken wire to prevent livestock and wildlife from eating the seeds and seedlings. A transparent plastic sheet was added for protection in case of rain or low night
Fig. 1 Map of Guatemala showing the three communities where the experiment was carried out and the location of the Huehuetenango Department within Guatemala (shaded area). Chiantla and Aguacatán are two municipalities of Huehuetenango.
temperatures. In the initial germination test, three replicates of 100 untreated seeds were randomly selected (no selection for undamaged seeds was carried out for the viability tests in sand benches) and sown in each of the three sand benches. Before sowing, seeds were visually scored as being intact or damaged by insects and/or fungi. After 3 and 6 months of storage in the community (both in the farmer’s house and CSR), 100 seeds from each container were randomly selected, visually sorted into intact and damaged categories, and then sown in the sand bench. In all of the seedling emergence experiments, all the replicates of 100 seeds each were sown in random order in individual mini-plots in the sand bench. Each plot was separated by a few centimeters from the others and from the edges of the sand bench. Seedling emergence was observed every 3 days. The duration of the

Fig. 2  a The three conservation treatments of the drying experiment (6 flasks plus 3 mesh bags) on the uppermost shelf in the CSR of San Francisco. In the lower shelves, part of the horticultural crops and maize seed collections of the reserve can be seen.  b The flasks and the mesh bags of the three conservation treatments in the farmer’s house in Climentoro, in the same room where the farmer stores his own seeds.  c The complete set of flasks and mesh bags of the experiment from the CSR of San Francisco at the 6-months time point. The humidity indicator strips in the RH indicator cards were pink in the mesh bags and flasks containing untreated seeds (RH > 75%) and blue in the flasks with dried seeds (RH < 35%).
seedling emergence experiments was variable in the different communities and times of the year due to the different climatic conditions experienced by the seeds and seedlings: at 15, 20 and 50 days post-sowing for Climentoro; 11, 16 and 17 days for Quilinco, and 11, 14 and 18 days for San Francisco for the initial, 3- and 6-months experimental time points, respectively. At the completion of the seedling emergence tests, all of the seedlings in the dried seed treatment had reached the 3-leaf stage. At this point, 20 randomly selected seedlings per plot were harvested and total lengths of the roots and shoots (up to the first leaf) were measured. Seedling size indirectly reflects time of emergence, as seedlings that emerge earlier have longer growing times before harvest.

**Germination in the ICTA germination laboratory**

At all three time points (initial, 3-months and 6-months), germination experiments were performed at ICTA facilities. The internal protocol for seed testing at ICTA was employed for these tests: 50 seeds that appeared intact, not infested nor damaged in any way, were selected for the test. Seeds were sterilized by soaking them in 2% commercial bleach for 3 min and then rinsed two times in water. Sterilized seeds were sown in rolled, moistened paper towels and placed in an incubator (Biotronette, Plant Growth Chamber, Lab-Line) set at 30°C/20°C at 8/16 h (light/dark) photoperiod for 7 days. At the initial time point, three replicates of 50 seeds were tested. At the 3- and 6-months time points, 50 seeds from each of the 54 treatment containers in the experiment were tested for germination.

**Statistical analyses**

The germination, seedling emergence and seed quality data were analyzed by generalized linear models (GLM) with binomial distribution and logit as link function. The results of seedling emergence and growth in sand benches at the different time points were not compared across locations since the environmental conditions varied considerably across communities and times of the year. The seedling measurements were analyzed with a GLM with normal distribution and identity as link function. Moisture content (MC) data were analyzed with a GLM with gamma distribution and log as link function. Pairwise comparisons were employed to compare the different treatments. All analyses were performed using SPSS for Windows, version 21.0.

**Results**

**Prior to storage**

**Drying, seedling emergence and germination**

After eight days of drying using drying beads, the seed moisture content (MC) decreased from an initial value of 18.4 ± 0.2% to 11.5 ± 0.3% (mean ± st.dev., fresh weight basis). No significant differences were detected in terms of MC between seeds dried in the two hermetic boxes (Wald chi-square = 1.114, df = 1, p = 0.291).

The initial germination percentage of the seeds before the application of the treatments was 85.3 ± 2.9% in Climentoro, 86 ± 6.1% in Quilinco, 81 ± 5.5% in San Francisco in the sand benches, and 88 ± 3% in the ICTA incubator.
After three months’ storage

Seedling emergence in sand benches

After 3 months of storage, the final seedling emergence tested in the sand benches differed significantly across the three communities and was significantly affected by the treatments (Fig. 3; Table 1). On the other hand, the site (farmer’s house vs CSR) where the seeds were stored did not have a significant effect on emergence. The magnitude of the effect of the treatments was significantly different in the different communities; the other interactions were not significant (Table 1). In all three communities, the highest final seedling emergence (always > 89%; Fig. 3) was achieved by seeds dried with beads and conserved in hermetic containers (Beads + Herm.Flask). In the community at the highest altitude (Climentoro), the lowest final emergence was by seeds conserved in mesh bags (72 ± 7%; Fig. 3), while in the communities at lower altitudes (Quilinco and San Francisco), the untreated seeds conserved in hermetic containers showed the lowest seedling emergence (55 ± 7% and 53 ± 10%, respectively; Fig. 3).

Seedling lengths

Community and treatment had significant effects on the length of the seedlings at the 3-months time point (Fig. 4; Table 1). Also, the effect of site (farmer’s house versus CSR) was slightly significant because in Climentoro seedlings from seed conserved in the farmer’s house grew slightly longer than those stored in the CSR (p < 0.001), with mean seedling lengths of 160 and 150 mm, respectively. The interactions between community and treatment and site and treatment were also significant (Table 1). In all communities, the seedlings developed from seeds dried with beads (Beads + Herm.Flask) were
significantly longer (p < 0.05). The untreated seeds conserved in mesh bags and in hermetic containers did not differ in seedling length (p > 0.05; Fig. 4).

After six months’ storage

Seedling emergence in sand benches

At the end of the experiment (6 months after the seed treatment sets were placed in the communities), the differences in final seedling emergence in the sand benches among the three communities were highly significant (Fig. 3; Table 1). The effects of the treatments and sites as well as all of the interactions among them were also significant (Table 1). In all the communities, the highest seedling emergence was achieved by seeds previously dried with drying beads and conserved in hermetic flasks (> 80%; Fig. 3). The difference in final emergence between seeds conserved in the two different sites (farmer’s house versus CSR) was significant in San Francisco (Wald chi-square = 45.074, df = 1, p < 0.001) but not in Climentoro (Wald chi-square = 0.257, df = 1, p = 0.257) nor in Quilinco (Wald chi-

| Experiment                  | Factors             | Wald Chi-square | df | Sign  |
|-----------------------------|---------------------|-----------------|----|-------|
| Seedling emergence 3 months | Community           | 46.034          | 2  | < 0.001 |
|                            | Treatment           | 319.300         | 2  | < 0.001 |
|                            | Site                | 0.018           | 1  | 0.893  |
|                            | Community*Treatment | 51.258          | 4  | < 0.001 |
|                            | Site*Treatment      | 3.112           | 2  | 0.211  |
|                            | Community*Site      | 3.257           | 2  | 0.196  |
| Seedling length 3 months   | Community           | 431.643         | 2  | < 0.001 |
|                            | Treatment           | 39.676          | 2  | < 0.001 |
|                            | Site                | 4.204           | 1  | 0.040  |
|                            | Community*Treatment | 14.268          | 4  | 0.006  |
|                            | Site*Treatment      | 9.884           | 2  | 0.007  |
|                            | Community*Site      | 0.137           | 2  | 0.934  |
| Seedling emergence 6 months| Community           | 132.211         | 2  | < 0.001 |
|                            | Treatment           | 738.203         | 2  | < 0.001 |
|                            | Site                | 12.439          | 1  | < 0.001 |
|                            | Community*Treatment | 367.872         | 2  | < 0.001 |
|                            | Site*Treatment      | 13.558          | 2  | 0.001  |
|                            | Community*Site      | 44.053          | 1  | < 0.001 |
| Seedling length 6 months   | Community           | 808.038         | 2  | < 0.001 |
|                            | Treatment           | 55.669          | 2  | < 0.001 |
|                            | Site                | 14.525          | 1  | < 0.001 |
|                            | Community*Treatment | 188.115         | 4  | < 0.001 |
|                            | Site*Treatment      | 14.205          | 2  | 0.001  |
|                            | Community*Site      | 33.741          | 2  | < 0.001 |
square = 0.146, df = 1, p = 0.703). Climentoro, the highest altitude site, was the community in which the untreated seeds achieved the highest seedling emergence (71 ± 9%) without significant differences between seed conserved in mesh bags and hermetic flasks (p = 0.878; Fig. 3). The seeds dried with beads achieved a significantly higher emergence percentage (83 ± 5%, p < 0.001; Fig. 3). In Quilinco, the untreated seeds conserved in the hermetic flasks had the lowest seedling emergence (17 ± 7%, p < 0.001). Seeds conserved in mesh bags achieved a final emergence of 69 ± 3%, a significantly lower value than seeds dried with beads and conserved in the hermetic flasks (88 ± 3%, p < 0.001; Fig. 3). In San Francisco, a statistical difference in seedling emergence was detected between sites (farmer’s house versus CSR) for the undried seeds conserved in mesh bags and flasks (p < 0.001), but not for the dried seeds conserved in hermetic flasks (p = 0.464) which achieved the highest seedling emergence (> 90%, Fig. 3) in both sites. On the other hand, the untreated seeds conserved in mesh bags and hermetic flasks had a higher seedling emergence when conserved in the CSR rather than in the farmer’s house: 45 ± 5% and 18 ± 10% respectively for mesh bags and 42 ± 5% and 19 ± 4% respectively for hermetic flasks (Fig. 3).

Seedling lengths

The main effects of community, treatment and site were significant for seedling growth at the 6-months time point, as were the interactions among them (Fig. 4; Table 1). Climentoro was the only community in which there were no statistical differences in terms of seedling length among treatments (Wald chi-square: 3.767, df = 2, p = 0.152). In Quilinco, the untreated seeds conserved in hermetic flasks developed significantly shorter seedlings (p < 0.001) while there was no statistical difference in terms of seedling length between seeds conserved in mesh bags and seeds conserved in hermetic flasks and previously dried with drying beads (Beads + Herm.Flask, p = 0.739; Fig. 4). In San Francisco, the
difference in seedling growth between the two sites (CSR and farmer’s house; Wald chi-square: 36.413, df = 1, \( p < 0.001 \)) was statistically significant, while it was not in Quilinco (Wald chi-square = 0.209, df = 1, \( p = 0.648 \)). In San Francisco, the longest seedlings were the ones developed from seeds (either dried or undried) conserved in hermetic flasks. Storage in the CSR had a positive effect in terms of seedling growth for the seeds conserved in mesh bags (\( p < 0.001; \) Fig. 4).

**Climatic conditions**

In all communities, the relative humidity was always high (65–90%) throughout all 6 months of the experiment, with higher values in the CSR (Table 2). Climentoro, the highest altitude community, was clearly the coldest site overall (Table 2). In all communities, the temperatures in the CSR were on average lower than in the farmer’s house. In Climentoro and Quilinco, the differences between day and night temperatures were more marked in the farmer’s house than in the CSR (on average 3.5 °C vs 0.7 °C in Climentoro and 3.1 °C vs 0.7 °C in Quilinco). On the other hand, in San Francisco there was little difference between CSR and farmer’s house in day and night temperatures (on average 3.2 °C in the CSR and 3.4 °C in the farmer’s house, Table 2).

**Seed moisture content**

The initial moisture content of the untreated seeds at the beginning of the experiment was 18.4 ± 0.2%. This was reduced to 11.5 ± 0.3% in seeds dried with drying beads. These MC did not change significantly during subsequent storage in the hermetic flasks in any of

| Community       | Site     | Months 1-3       | Months 4-6       | Average          |
|-----------------|----------|------------------|------------------|------------------|
| Climentoro      | House    | 16.1 / 12.6 °C   | 15.8 / 12.4 °C   | 16 / 12.5 °C     |
| (2959 m a.s.l.) | CSR      | 13.6 / 12.8 °C   | 12.6 / 11.9 °C   | 13 / 12.3 °C     |
|                 |          | 13.2 °C, 83.3%   | 12.3 °C, 90.3%   | 12.7 °C, 87.9%   |
| Quilinco        | House    | 19.7 / 16.3 °C   | 18.6 / 15.9 °C   | 19.2 / 16.1 °C   |
| (2452 m a.s.l.) | CSR      | 17.1 / 16.6 °C   | 17.1 / 16.1 °C   | 17 / 16.5 °C     |
|                 |          | 16.9 °C, 75.2%   | 16.6 °C, 80.5%   | 16.7 °C, 77.9%   |
| San Francisco   | House    | 21 / 17.1 °C     | 20 / 16.4 °C     | 20.1 / 16.7 °C   |
| (1985 m a.s.l.) | CSR      | 19.9 / 16.4 °C   | 18.5 / 15.4 °C   | 19.1 / 15.9 °C   |
|                 |          | 18.3 °C, 79.2%   | 16.9 °C, 85.3%   | 17.6 °C, 82.2 %  |

Data from the first and last 3 months (May–August and August-November) are pooled together separately by interval (Months 1–3 and 4–6), and in the last column (“Average”) mean values for the entire duration of the experiment are presented. In each cell in the upper row are presented the mean daily temperature (in red) and mean night temperature (in blue). In the lower row the mean temperature (in black) as well as the mean relative humidity (in green) are presented.
the communities and sites (Wald chi square: 0.993, df = 2, p = 0.609; Table 3). Significant changes in MC across data collection points were detected in untreated seed conserved both in hermetic flasks and in mesh bags (Wald chi square: 16.600, df = 2, p < 0.001 and Wald chi square: 13.430, df = 2, p = 0.001; Table 3). In particular, the MC of untreated seeds conserved in hermetic flasks increased significantly between the 3- and 6-months data collection points (p < 0.001, Table 3). On the other hand, the MC of untreated seeds conserved in mesh bags was significantly different between the initial and the 3-months time point (p = 0.001).

**Germination tests in the laboratory (ICTA)**

In tests conducted in the incubator of the ICTA genebank, differences in germination percentages were significant across communities, treatments and time-points, but not between sites (farmer’s house versus CSR) (Fig. 5; Table 4). Except for Treatment * Site and Site * Time, all the interactions among factors were significant (Table 4). In all communities, the seeds dried with beads maintained very high germination percentages (> 90%, Fig. 5), not statistically different from the initial values (p > 0.05). On the other hand, in all communities, the germination of the untreated seeds significantly decreased over time (p < 0.05), except for seeds conserved in mesh bags in Quilinco in the last data collecting point (p = 0.262, Fig. 5).

| Community  | Site  | Treatment                | MC (% ± s.d.) 3 months | MC (% ± s.d.) 6 months |
|------------|-------|--------------------------|------------------------|------------------------|
| Climentoro | House | Mesh bag*                | 14.9 ± 0.05            | 15.5 ± 0.05            |
|            |       | Hermetic flask           | 17.3 ± 0.06            | 17.5 ± 0.15            |
|            |       | Beads + Herm.Flask       | 11.8 ± 0.1             | 11.6 ± 0.8             |
| CSR        |       | Mesh bag                 | 17.9 ± 0.2             | 18.1 ± 0.05            |
|            |       | Hermetic flask           | 17.5 ± 0.15            | 17.6 ± 0.05            |
|            |       | Beads + Herm.Flask       | 11.8 ± 0.2             | 11.7 ± 0.4             |
| Quilinco   | House | Mesh bag*                | 14.7 ± 0.4             | 16 ± 0.4               |
|            |       | Hermetic flask*          | 17.9 ± 0.3             | 19.1 ± 1               |
|            |       | Beads + Herm.Flask       | 11.6 ± 02              | 11.6 ± 0.3             |
| CSR        |       | Mesh bag*                | 15.3 ± 0.2             | 16.6 ± 0.3             |
|            |       | Hermetic flask           | 17.7 ± 0.6             | 18.2 ± 0.3             |
|            |       | Beads + Herm.Flask       | 11.6 ± 03              | 11.6 ± 0.5             |
| San Francisco | House | Mesh bag                 | 15.4 ± 0.5             | 15.1 ± 0.3             |
|            |       | Hermetic flask*          | 17.3 ± 0.2             | 19.3 ± 0.4             |
|            |       | Beads + Herm.Flask       | 11.6 ± 02              | 11.7 ± 0.2             |
| CSR        |       | Mesh bag*                | 16.6 ± 0.6             | 17.4 ± 0.2             |
|            |       | Hermetic flask*          | 17.3 ± 0.1             | 18.5 ± 0.3             |
|            |       | Beads + Herm.Flask       | 11.6 ± 03              | 11.8 ± 0.2             |

Asterisks indicate a significant difference (p < 0.05) in MC between the two time points.
Three months after the beginning of the experiment, all of the undried seeds in mesh bags and flasks showed a significant decline in final germination ($p < 0.05$).

In Climentoro, at the end of the experiment (6-months time point), untreated seeds conserved in both mesh bags and hermetic flasks showed a significant decline in final germination ($p < 0.001$), and to a similar extent (average final germination = 63 ± 16%; $p = 0.130$; Fig. 5). The lowest germination levels were reached by untreated seeds conserved in hermetic flasks in Quilinco and San Francisco (37 ± 14% and 36 ± 7% respectively, Fig. 5).

**Seed quality**

The quantities of intact seeds (without visual evidence of infestation by fungi and/or insects) differed significantly across communities, treatments, time points and between sites (Fig. 6; Table 4). All the interactions between factors were significant with the exception of Treatment*Site and Site*Time (Table 4). In all communities, the percentage of intact untreated seeds in both mesh bags and hermetic flasks decreased significantly over time, in most of the cases already decreasing after only 3 months ($p < 0.05$). On the other hand, seeds dried with beads and conserved in hermetic flasks always maintained high quality (percentage of intact seeds > 98%, Fig. 6) in all of the time points, communities and sites considered ($p < 0.001$) (Fig. 6).

In Climentoro, despite significant differences in seed quality among time points ($p = 0.002$), the percentage of intact seeds was always > 90% (Fig. 6). In Quilinco, the percentage of intact kernels was also > 90%, with the exception of untreated seeds conserved in hermetic flasks, in which the percentage dropped to 74 ± 19% by the 6-months time point, with 18% of the seeds infested by fungi and 8% by insects. San Francisco was the only community in which a significant difference between sites (farmer’s house and CSR) was detected in terms of seed quality, and only at the 6-months time point (Wald Chi-Square: 160.065, df = 1, $p < 0.001$). The lowest percentages of intact seeds at the end of the experiment were detected in San Francisco in the untreated seed in mesh bags and flasks conserved in the farmer’s house, 32 ± 7 and 62 ± 5% respectively. In particular, for the seeds conserved in mesh bags, 4% of the seeds were affected by fungi and 63% by insects, while for the untreated seeds conserved in flasks, 30% of the seeds were affected by fungi and 8% by insects. On the other hand, at the 6-months time point in San

![Fig. 5](https://example.com/figure5.png)

**Fig. 5** Final germination in ICTA incubator at the three time points (initial, 3-, and 6-months) of the experiment in the three different communities for the three different treatments. One asterisk indicates a significant difference from the initial germination, two asterisks indicate that the germination at the 6-months time point was statistically different from the value at 3-months in the same treatment. Asterisks indicate differences significant at $p < 0.05$
Discussion

Our results demonstrated that implementation of the dry chain concept, which refers to the initial desiccation of seeds to safe MC levels followed by storage in hermetic containers, is an optimal strategy for the conservation of viable seeds. Furthermore, we show that it succeeds in tropical and humid environments where seeds are susceptible to rapid losses of germination capacity.

In all of the participating communities, and in both CSRs and farmer’s houses, wherever maize seeds were dried with zeolite beads and stored in hermetic flasks, their viability (measured as seedling emergence in a sand bench or as seed germination in an incubator) did not decline across time and remained > 80% in all tests. Seeds dried with drying beads also maintained extremely high quality: > 95% of the seeds remained fungi- and insect-free for the 6-month duration of the experiment. In some of the seedling emergence experiments, the treatment with beads followed by storage in hermetic flasks significantly preserved the vigor of the seedlings, in terms of seedling length, compared to the other storage conditions. Seed ageing, accelerated by high seed moisture content, is indeed
known to detrimentally affect not only seed germination but also seedling vigor (Al-Maskri et al. 2003).

On the other hand, seed viability and quality of the untreated seeds declined drastically in most of the combinations of environments (sites and containers) after only 6 months of storage. The viability of untreated seeds stored in either mesh bags or hermetic flasks decreased significantly after 3 months in storage and, in most of the cases, continued to decline even more by 6 months. The worst results in terms of seed viability (seedling emergence < 20% and germination < 40%) were obtained from undried seeds in hermetic flasks in the localities at lower altitudes (Quilinco, San Francisco). These were the sites with warmer ambient temperatures that would contribute to the detrimental effects of infestations and seed ageing (Dickie et al. 1990; Mendoza et al. 2016). Consistently, the percentage of intact seeds significantly decreased over time in the untreated samples, in most of the cases already at the 3-months time point, with the lowest final value of only 32% of intact seeds in mesh bags at the farmer’s house in San Francisco.

The low viability and high degree of infestation of high moisture seeds conserved in hermetic flasks are particularly worrisome since this is the conservation method currently employed by the CSRs. These results highlight that if seeds are not properly dried, conservation in a hermetic flask can even be counterproductive, as the maintenance of very high moisture content in the closed container increases seed ageing and promotes infestations at a very high rate (Ellis and Roberts 1980; Dickie et al. 1990; Welty et al. 1987; Pittendrigh et al. 2003). This can ultimately lead to rapid loss of many seed accessions of local agrobiodiversity stored in the CSRs as well as greatly reduce the availability of quality seeds that the farmers store in the CSRs as a safety backup (Fuentes 2018).

Considering these results for seed viability, as well as the very high relative humidity in these communities (> 70%) and in the flasks containing seeds (> 75%), we can confirm that the very high moisture of the seed accessions, leading to a very rapid loss of seed viability, is the greatest threat to seed conservation in the CSRs. This problem can only be solved by providing a uniform drying regimen for all accessions stored in the CSRs as a safety backup (Fuentes 2018).

Drying beads achieved rapid drying of the maize grains, reducing their MC from 18.4 to 11.5% during only one week of treatment without heating. The seed MC could have been
lowered even further, closer to the international standards for long-term conservation (< 8% of seed moisture content; Rao et al. 2006) by reactivating and replacing the beads every 1–2 days. As the drying beads can be reactivated repeatedly, a relatively small quantity of beads along with an oven for reactivation can be used to dry a large quantity of seeds over time in a CSR. Subsequent storage of the dry seeds in hermetic containers then maintains the dryness and extends seed longevity without the need for further inputs such as refrigeration. Despite the high relative humidity inside the CSRs, the moisture content of dried seeds in the flasks in which the seeds were stored did not vary significantly over time. This demonstrates that the flasks employed by the CSRs are indeed hermetic. The air-tightness of the flasks is important not only to maintain the moisture content of the dried seeds but also to reduce the oxygen available for insects and fungi and for oxidation of seed components associated with ageing (Baoua et al. 2014; Groot et al. 2014; Williams et al. 2017a, b).

Overall, seed conservation in a CSR showed some technical advantages over traditional seed storage in farmers’ homes. First of all, the climatic data recorded by the data-loggers showed that average ambient temperatures were always lower in the CSRs than in farmers’ houses, which would tend to extend seed longevity. Additionally, in Climentoro and Quilinco, temperatures in the CSR were more constant than in the farmer’s house; relatively high temperatures and temperature fluctuations are known to detrimentally affect seed lifespan as well as increase fungal and insect infestations (Dickie et al. 1990; Mendoza et al. 2016). This is reflected in the results for seed quality and seedling emergence in San Francisco after 6 months of storage: the untreated seed conserved in the CSR showed a significantly higher seedling emergence, seedling length and percentage of intact seeds. This is probably due to the lower temperatures experienced by the seeds in the CSR and to the pest control periodically carried out in the CSR that reduced the amount of seeds in open storage damaged by insects. These data underscore the suitability of the CSRs of the study area as centers for agrobiodiversity conservation as well as storage locations for backups of farmers’ seeds.

It is interesting to note that the untreated seeds conserved in the community located at the highest altitude (Climentoro, 2959 m.a.s.l.), and consequently experiencing the lowest environmental temperatures, maintained on average a higher viability and percentage of intact seeds when compared to the other two locations located at lower altitudes. This can be explained by the fact that the lower temperatures experienced by the seeds limited seed ageing and infestations (Dickie et al. 1990; Mendoza et al. 2016). Duplication of seed collections, i.e., storing the same accession in a second location to provide insurance against loss of material, is of key importance in seed conservation (Rao et al. 2006). In the network of seed reserves of the Sierra de los Cuchumatanes, the CSRs at the highest altitudes that experience lower environmental temperatures, like Climentoro, could be designated as locations to store safety-duplicates of seed collections from other communities in the network at lower altitudes in order to prevent the loss of those genetic resources.

The high viability and quality of the seeds dried with beads and conserved in hermetic storage in farmers’ granaries show that the dry chain can be applied to improve seed conservation also in farmers’ storage. High seed moisture content is the major threat to seed security in the study area (Mendoza et al. 2016), so providing farmers access to drying methods and hermetic containers could have major impacts. The dry chain utilizing reusable desiccants presents an efficient drying system that can enhance seed security, and, in the process, promote resilience and improve livelihoods for smallholder farming communities throughout the world.
Our experiments in Guatemala demonstrate also that the dry chain is an excellent, versatile solution for seed drying and conservation in humid and tropical environments. This is of particular importance considering that critical hotspots of plant biodiversity are located in tropical areas where seeds are susceptible to rapid losses of germination capacity. Recent research in Pakistan (Bakhtavar et al. 2019; Bakhtavar and Afzal 2020a, b) showed that the dry chain technology is effective in preserving seed viability and seed quality in maize, quinoa and wheat seeds, underscoring the versatility of this seed conservation methodology. The dry chain has the potential to greatly improve medium-term seed conservation in community seed reserves, as well as in regional seed banks in low- and middle-income countries, where power-consuming conservation technologies are unavailable.

Based on the positive results of the current experiment, the dry chain is being implemented in the CSR of Quilinco to enhance the conservation of maize and bean seed accessions, with plans to scale out to dry the already existing seed collections in the entire CSR network as well as to implement initial drying for all of the new accessions coming into the reserves. Seminars and webinars, directed at Guatemalan farmers and agricultural professionals, are being carried out to disseminate the results of this research project and the potential of the dry chain concept. While this technology may still be economically inaccessible for individual farmers, one of the future aims of this project is to provide each CSR with a drying kit based on the dry chain concept. In this way, the CSRs could also serve as “drying centers” for the farmers of the area, who can bring their seeds to the CSR to be dried and then conserved in hermetic containers, either at the CSR or in their own homes or, ideally, in both sites (Dadlani et al. 2016; Bradford et al. 2018). Reactivation of beads by heating at > 200 °C in an oven for two hours (Kunusoth et al. 2012) has been successfully carried out in Quilinco with a gasoline generator-powered oven. Thus, reactivation can be achieved even in communities with no access to an electrical power grid.

Further research is ongoing to combine the dry chain technology described here with the “blue drums”, a drying protocol developed by the Millennium Seed Bank of the Royal Botanic Gardens (Kew, UK), consisting of a sealable, moisture-proof plastic drum containing a desiccant agent (commonly silica gel) placed within a central cone made of metallic net from which to hang bags of seed collections for drying (Sutcliffe and Adams 2014). These drums are already being used successfully as a low-tech drying method in seed banks in 47 different countries and territories across the globe (Rossi et al. 2014; Martens 2018). In the CSR of Quilinco, tests are underway using locally manufactured drums plus drying beads in order to dry several kilos of seeds at once.

The high moisture content of maize grains (the staple of western Guatemala) in farmer’s storage, ranging from 17 to 20% in the study area (Mendoza et al. 2017) often causes increases in mycotoxin occurrence (e.g., fumonisin and aflatoxins) that are responsible for severe health effects such as liver necrosis and tumors, depressed immune esophageal cancer, stunting and neural tube defects (Bryla et al. 2013; Wu et al. 2014). Bakhtavar and Afzal (2020b) demonstrated that the dry chain technology prevents increases in aflatoxin levels in wheat grain during storage. Further research is needed to test the application of the dry chain technology, and in particular drying beads, for grain drying to reduce post-harvest losses and, in particular, reduce mycotoxin occurrence in maize grains in Guatemala and other tropical areas (Blakeney 2019).

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**Compliance with ethical standards**

**Conflict of interest** The use of any commercial product does not constitute an endorsement by CIMMYT. The authors have no conflict of interest.

**Ethical approval** The research protocol followed in this research was approved by CIMMYT’s Internal Research Ethics Committee (IREC-2020.008).

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