# Is It Still Necessary to Continue to Collect Crop Genetic Resources in the Mediterranean Area? A Case Study in Catalonia

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## Abstract

Is It Still Necessary to Continue to Collect Crop Genetic Resources in the Mediterranean Area? A Case Study in Catalonia Crop genetic resources have been extensively collected in Europe in the last century, creating large, publicly available *ex situ* collections. While this huge genetic diversity is often underutilized, in recent decades, several initiatives have emerged at the local level to collect germplasm cultivated on farm. Uncoordinated actors often carry out these collecting missions without considering previously collected data. To explore whether new collecting missions are likely to be worthwhile, we studied the crop genetic resources conservation network in Catalonia by analyzing the passport data and geographical distribution of germplasm stored in seed banks. Moreover, to determine whether this germplasm was representative of the diversity cultivated on farm, we performed new collecting missions in four randomly selected areas in the European Union's Natura 2000 network and compared the results with the *ex situ* databases. Seed banks currently hold a large germplasm collection (2931 accessions), although most materials are conserved in private collections without regulated systems for seed regeneration and are not present as duplicates in the National Inventory. One important shortcoming of the *ex situ* network is that the germplasm conserved *ex situ* shows a low geographical coverage, representing only 35.3% of the municipalities in Catalonia. Our new missions allowed us to collect 234 accessions, mostly tomatoes (17.5%) and beans (16.2%). The ecological indicators' richness (both at species (S) and variety (V) levels), total abundance (A), and the Shannon-Weaver diversity index calculated at species (H2, considering the different accessions of each variety as a single population) and variety levels (H3, considering the intra-varietal genetic diversity) were higher in the newly collected germplasm than in the *ex situ* collections, suggesting that seed banks do not accurately represent the genetic diversity still cultivated on farm. Moreover, some important landraces from each area were absent or underrepresented in the *ex situ* collections. Thus, it is necessary to continue to devote efforts to collecting germplasm; better organization between actors and targeting specific species/varieties can increase the efficiency of new collecting missions. As a conclusion, we propose different criteria to guide new missions and to improve the network's conservation activities.

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## Key Words

Landrace
horticultural crops
agrobiodiversity
*ex situ* conservation
on-farm conservation
germplasm bank
ethnobotany.

# Introduction

Conservation of crop genetic resources (CGR) is one of the most important challenges that agriculture has faced in the last century. CGR are essential for agricultural resilience and sustainability and can play a key role in adapting crops to changing climates (Gepts 2006). In the last century, agricultural ecosystems and production methods changed drastically, leading to the replacement of historically cultivated varieties by modern varieties with higher performances (van de Wouw et al. 2010) and the loss of many varieties (i.e., genotypes). In Europe, it is estimated that 70 to 100% of the genotypes have been lost, depending on the crop and the area studied (reviewed by Hammer and Teklu (2008)). The international community reacted to this threat to sustainability, promoting the International Treaty on Plant Genetic Resources for Food and Agriculture [ http://www.fao.org/plant-treaty/en/ ] (verified 6 December 2016).

In parallel, efforts have been made to conserve CGR by collecting landraces (i.e., varieties characterized by distinctive agromorphological traits and linked to traditional dishes in their areas of origin) (Zeven 1998), (old) folk varieties (Berg 2009), underutilized crops (Galluzzi and López Noriega 2014), and crop wild relatives (Maxted et al. 2007). Initially, the strategy was to collect the maximum genetic diversity in as many ecogeographical areas as possible, accumulating a large number of accessions in seed banks. In recent years, scientist have started to review this huge amount of information, compiling data from different collection missions in attempt to understand genetic richness and improve passport data (Tanksley and McCouch 1997; Thomas et al. 2012; Thormann et al. 2012), developing new collection missions to collect specific landraces still not present in the *ex situ* collections (Laghetti et al. 2005), documenting the degree of actual on farm conservation (Jarvis et al. 2008; Montesano et al. 2012; Portis et al. 2012), and comparing data from different collection expeditions to estimate genetic erosion (Hammer et al. 1996; Hammer and Laghetti 2005). Promoting in situ conservation (preservation of traditional agrosystems and empowerment of farmer networks that conserve seeds) in combination with *ex situ* conservation tools has proven the best strategy to preserve genetic diversity and maintain its evolution (Gepts 2006).

Molecular technologies offer promising tools to understand what is filed in seed banks, rationalize the germplasm collections, and detect genetic variants with agronomic interest (Prada 2009). Meanwhile, germplasm collection missions continue to be conducted in scarcely studied areas, but also in areas where collection activity has been intense in the last 70 years. This implies that important financial resources are still devoted to collecting CGR, so it is worth analyzing whether new expeditions in certain areas make sense. This will enable stakeholders and scientists to make decisions about what is still necessary to collect from where and about what should be conserved. Furthermore, various actors (governments, universities, nonprofit organizations, and botanical gardens) are involved in CGR conservation, and it is important to understand their relative contributions to CGR conservation to increase the efficiency of this network.

The Mediterranean basin is remarkable for its CGR (Veteläinen and Maxted 2009). In addition to being the original site of some domestications, the basin has been a hotspot for the introduction and diversification of new crops and is considered a secondary center of diversification for many crop species (Hancock

2004). The co-evolution of both native and newly introduced crops with the diversity of agrosystems and food cultures found in this area, directed by farmer "field plant breeding," has originated a wide diversity of (old) folk varieties and local landraces. Although genetic erosion has drastically diminished the genetic variability, a significant diversity of forms is still present, mostly in non-intensive agrosystems managed by old farmers (Calvet-Mir et al. 2011; Montesano et al. 2012). Some landraces, mainly those with distinctive morphological traits and sensory profiles, are still produced in large quantities to sell in the markets.

Catalonia, in NE Spain, has a surface area of 31,895 km$^2$ and 3 climate types (Mediterranean, alpine, and oceanic). Agricultural activity has been historically important in this region, covering at present 36% of the surface area. Catalonia is rich in CGR, with many references to landraces found in cookbooks and agricultural books, and some of these landraces are economically important (e.g., "Calçot de Valls" (*Allium cepa* L.) (Simó et al. 2012), "Penjar" tomato (*Solanum lycopersicum* L.) (Casals et al. 2012), or "Ganxet" bean (*Phaseolus vulgaris* L.) (Casañas et al. 1999)). Significant efforts have been made to collect local germplasm, with a National Spanish Inventory hosting hundreds of accessions from this area [ http://wwwx.inia.es/coleccionescrf/ ] (verified 6 May 2016). Boosted by increasing social awareness of the threats of genetic erosion, in recent years, several CGR-conservation initiatives have emerged at the local level, mainly aiming to use this agrobiodiversity to enhance the distinctiveness of local activities (both agriculture and food services). However, rather than taking advantage of previously collected germplasm, new collecting missions have been undertaken, mainly with funding from regional administrations without involving agricultural institutions, so the priorities and outcomes of these missions have been haphazard. For these reasons, Catalonia is a good place to analyze the activities around CGR conservation to examine how the efficiency of CGR conservation networks might be improved. Thus, we analyzed the genetic richness of *ex situ* collections and the relative contribution of each actor involved in CGR conservation. Furthermore, we carried out an experiment in four areas to estimate whether new germplasm collecting missions are still necessary.

# Materials and Methods

## Analysis of the *Ex Situ* Conservation Network in Catalonia

To compile information about CGR previously collected in Catalonia and conserved *ex situ*, we screened the National Inventory of Genetic Resources at the Instituto Nacional de Investigaciones Agrarias (INIA) to identify accessions of horticultural species (i.e., vegetables, pulses, and condiments) from the area of study. In Catalonia, nonprofit organizations have carried out several initiatives at the local level to collect and conserve CGR. Data from these local banks were also obtained through personal interviews with germplasm managers (Esporus (Manresa), Les Refardes-Gaiadea (Mura), EcoLlavors (Girona), and SIGMA (Olot)). Furthermore, data from collections held by botanical gardens (Jardí Botànic Marimurtra (Blanes)) and Universities (University of Lleida (UdL) and Polytechnic University of Catalonia-Miquel Agustí Foundation (UPC)) were also included in the study. To enable joint analysis, we entered all data in a single database, standardizing the information from the different germplasm banks (name of the varieties, name of the municipalities, etc.). All institutions were also asked about complementary activities beyond collecting (characterization, seed regeneration, and supplying).

To analyze passport data using geographic information systems (GIS), each collecting site (name of the municipality) was associated with its corresponding geographic coordinates. To study the spatial distribution of *ex situ* germplasm collections, we constructed a datasheet with the total number of accessions per municipality. Geographical analysis was performed using ArcGIS® software by Esri.

## Study in Four Natura 2000 Areas to Evaluate the Efficiency of Prior Collection Missions

### Identification of Collecting Sites

Rather than using passport data from *ex situ* collections, to evaluate the efficiency of previous germplasm collection missions, we used the European Union’s Natura 2000 network as a neutral matrix for CGR accessions. Sites protected under Natura 2000 are distributed throughout the territory and represent a great diversity of ecosystems, most of which are closely interrelated to agricultural activity (Ostermann 1998). In Catalonia, 963,035 ha of land (30% of the total area of the region) is protected under Natura 2000. We chose four sites in different areas of Catalonia that represent different ecosystems: ES5110015 (Sistema prelitoral central), ES5140011 (Sistema prelitoral meridional), ES5140019 (Riu Gaià), and ES5130029 (Serres de Queralt i Els Tossals-Aigua d’Ora), all together 83,022 ha, of which 5000 ha are occupied by agriculture. ES5110015 is an inland mountainous area in the center of Catalonia where cereals are the major crop; this site includes territories from 21 municipalities with 37,962 inhabitants. ES5140011 is an inland mountainous area in southern Catalonia where non-irrigated fruit trees are the major crop; this site includes territories from 24 municipalities with 78,146 inhabitants. ES5140019 is a coastal mountainous area in the center of Catalonia where non-irrigated fruit trees and vineyards are the major crops; this site includes territories from 13 municipalities with 16,483 inhabitants. Finally, ES5130029 is a mountainous area in the foothills of the Pyrenees in northern Catalonia where cereals are the major crop; this site includes territories from 8 municipalities with 7842 inhabitants. The main habitats present in ES5110015, ES5140011, and ES5140019 sites are Mediterranean pine forests with endemic Mesogean pines, while in ES5130029 the main habitats are sub-Mediterranean pine forests with endemic black pines.

## New Collecting Missions

New collecting missions were carried out during 2011 in all the municipalities in each selected Natura 2000 site. To identify farmers who might be preserving traditional seeds, we contacted managers of cooperatives and other agricultural institutions (e.g., syndicates) and technicians from local administrations prior to field surveys. Field surveys lasted at least 3 days in each municipality. To collect information during the interviews with farmers, we adapted the passport registration form proposed by the On-farm Conservation and Management Working Group (European Cooperative Programme for Crop Genetic Resources) ([ http://www.ecpgr.cgiar.org/working-groups/on-farm-conservation/ ] (verified 5 December 2016)). The form includes sociological information about the germplasm owner (age, work), the seeds provided (name of the variety, antiquity, main uses, and recent exchanges of seeds with other farmers), and geographic coordinates of the collecting site.

## Data Analysis

To compare *ex situ* accessions conserved in germplasm banks with the accessions collected in our new missions, we adapted the ecological indicators proposed by Montesano et al. (2012) for existing databases and databases generated in the new collecting expeditions at each Natura 2000 site: species richness (S, number of collected species); variety richness (V, number of collected varieties); and total abundance (A, number of collected accessions). To calculate the degree of agrobiodiversity preserved by each system, we employed the Shannon-Weaver index, calculated using the formula $H = -\Sigma p_i \ln(p_i)$, where $p_i$ is the proportion of the *i*-th category on the total number of categories. As we had no prior information regarding the genetic integrity of each accession (no molecular or phenotyping data was available to study the genetic distance between accessions of the same variety), we used several approaches in order to calculate the index: (a) H1, considering species as the main category and accessions within species as the individuals used to evaluate the genetic diversity; (b) H2, considering species as the main category and varieties as the individuals used to estimate the diversity within species; and c) H3, considering the variety as the main category and accessions within each variety as the individuals used to evaluate the genetic diversity.

To identify significant differences between datasets, we performed analysis of variance (ANOVA) using the SAS statistical package (SAS Institute Inc. 1999).

# Results

## The *Ex Situ* Network

We identified 3568 accessions in germplasm banks: 1446 in the National Inventory and 2122 in other public and private collections (Table 1). However, 637 (27.9%) accessions in other public and private collections were duplicated in the National Inventory according to passport data, so there were 2931 unique accessions. The largest collection of accessions was the National Inventory ($n$ = 1446), followed by universities ($n$ = 1280) and nonprofit organizations ($n$ = 824); botanical gardens conserved few accessions ($n$ = 18). Tomatoes and dry beans were the most represented species, accounting for 1655 (56.5%) unique accessions conserved *ex situ* (Fig. 1). Other species with more than 100 accessions were different cultivar-groups of *Brassica oleracea* L., lettuces (*Lactuca sativa* L.), and peppers (*Capsicum annuum* L.). On the other hand, very few accessions of some historically important species were conserved *ex situ*; for example, there were only 6 accessions of carrots (*Daucus carota* L.), 5 of garlic (*Allium sativum* L.), and 5 of cardoon (*Cynara cardunculus* L.).

**Table 1.**

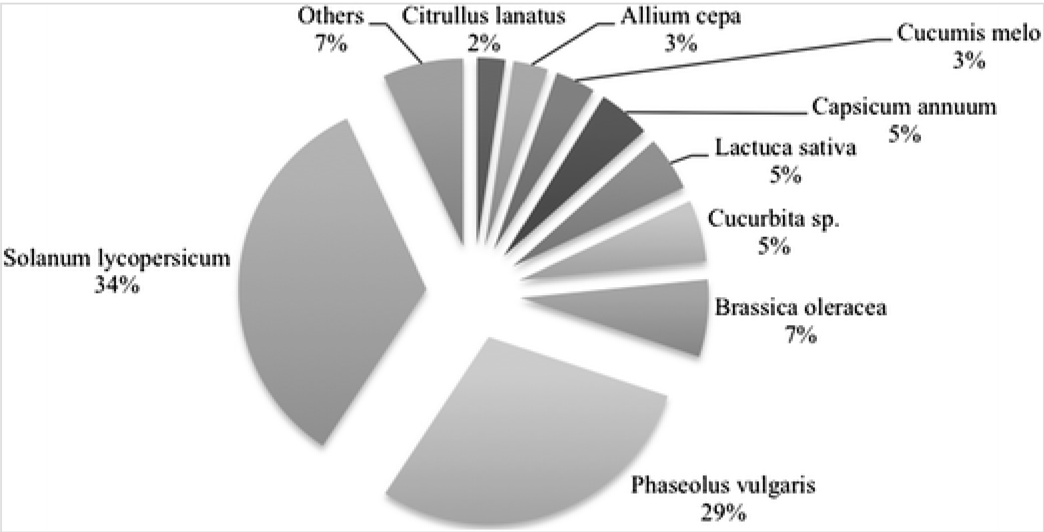
ANALYSIS OF THE *EX SITU* GERMPLASM CONSERVATION SYSTEM IN CATALONIA.

| Germplasm collection[a] | Number of accessions | Unique accessions | Duplicates from other public and private collections | Accessions with information about collecting site |
|---|---|---|---|---|
| National Inventory | 1446 | 809 | 637 | 94.6% |
| Public-private collections | 2122 | 2122 | - | 83.0% |
| TOTAL | 3568 | 2931 | 637 | 86.3% |

[a]National Inventory (INIA); public-private collections: data from germplasm banks held by universities, botanical gardens, and nonprofit organizations

**Fig. 1.**

Distribution by species in the *ex situ* collections.

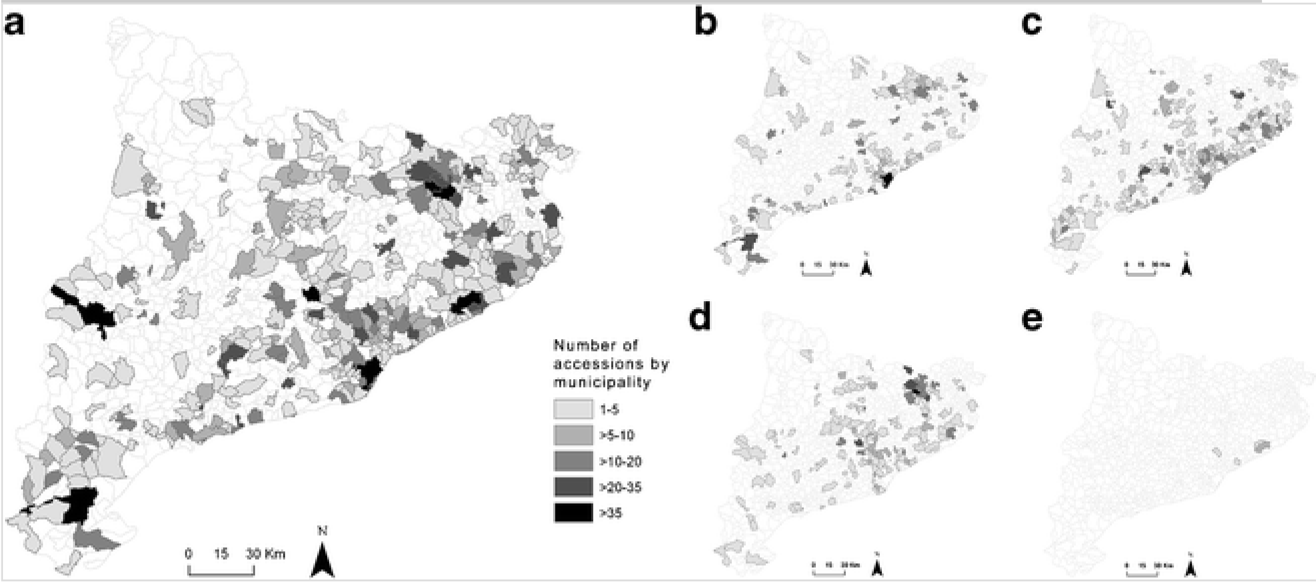



## Geographic Distribution of the Accessions and Link with the Activity of the Collecting Institutions

Geographical information about the collecting site was available in the passport data of 86.3% of the accessions conserved *ex situ*, enabling us to map the CGR conserved *ex situ* in Catalonia (Fig. 2). The CGR conserved in seedbanks is not well distributed across the territory: only 334 of 947 municipalities have 1 or more accessions conserved *ex situ* (geographical coverage, 35.3%) (Fig. 2a). In some areas, the number of accessions per municipality is unusually high (> 40 accessions), probably due to different factors: (a) the Polytechnic University of Catalonia-Miquel Agustí Foundation intensive germplasm collecting activities for plant breeding programs in specific landraces (e.g., Santa Pau bean in the Garrotxa district (Almirall et al. 2010), Ganxet bean in the Vallès Oriental and Vallès Occidental districts (Sanchez et al. 2008), and Montserrat and Pera Girona tomatoes in the Maresme and La Selva districts (Casals et al. 2010)); (b) the presence of a seedbank in the municipality (e.g., Manresa (54 accessions), where the Esporus seed bank is located); (c) or the presence of an important food market where accessions were collected (e.g., Barcelona, which has no agricultural activity but contributed 47 accessions). Germplasm collections at universities seem to have a higher geographical coverage (22.3%) (Fig. 2c) than those managed by nonprofit organizations (15.4%) (Fig. 2d) and those collected directly by the National Inventory (i.e., not duplicates sent by universities or nonprofit organizations) (14.5%) (Fig. 2b).

**Fig. 2.**

Geographic distribution of germplasm conserved *ex situ* in different seed banks: **a** entire germplasm collection (*n* = 2528 accessions; 86.3% with geographical data); **b** National Inventory (solely unique accessions are represented) (*n* = 765; 94.6% with geographical data); **c** universities (*n* = 1052; 82.2% with geographical data); **d** nonprofit organizations ($n$ = 696; 84.5% with geographical data); **e** botanical gardens ($n$ = 15; 83.3% with geographical data).




In addition to differing in their contributions to the geographic and genetic richness of the *ex situ* germplasm collections, actors in the CGR network differ in the other tasks related to conservation (Table 2). The National Inventory has the most secure collection, with a coordinate system of duplicates in different gene banks, equipment adapted for seed storage, and a program for seed regeneration. Universities are also secure, although they focus on specific species, so their contribution to the total CGR conservation system is narrower. Moreover, at universities, intense phenotyping activity ensures that the agricultural and morphological properties of the stored germplasm is well documented. Finally, nonprofit organizations lack security systems (equipment for seed storage, duplicates in different seed banks), so the stored seeds are not protected enough against genetic erosion; nevertheless, they play an important role in raising social awareness and in distributing seeds. So, the different contributions appear related to the different objectives of each institution.

**Table 2.**

Tasks performed by the different actors in the CGR network in Catalonia.

| Activity | National Inventory | Universities | Nonprofit organizations | Botanical Gardens |
|---|---|---|---|---|
| Collecting | All | Selective | All | No |
| Characterization | No | Intense | Partial | No |
| Seed regeneration | Yes | Partial | Partial | Yes |
| Security systems and optimal conditions of seed conservation | Yes | Yes | No | Yes |

| Activity | National Inventory | Universities | Nonprofit organizations | Botanical Gardens |
|---|---|---|---|---|
| Send duplicates to the National Inventory | – | Yes | No | No |
| Distribution of seeds | Free | No | Free | Free |

## The New Collecting Mission

A total of 234 accessions were collected in the four Natura 2000 areas studied (Table 3). The most common species collected were tomato (41 accessions, 17.5% of the total) and common bean (38 accessions, 16.2% of the total). Accessions from both species were collected in all the areas studied, signaling their broad adaptability (tomato germplasm was collected at sites between 15 and 925 m a.s.l., and common bean germplasm between 15 and 1215 m a.s.l.) and farmers' high esteem for landraces of these crops. Another important species collected was dry pea (29 accessions, 12.4% of the total) (*Pisum sativum* L.), mainly due to the presence of the "pèsol negre" (*black pea*) landrace in the mountainous ES5130029 area.

**Table 3.**

Number and frequency of the accessions collected in the four natura 2000 areas in 2011.

| Common name | Scientific name | Accessions (n) | Frequency (%) |
|---|---|---|---|
| Onion | *Allium cepa* L. | 3 | 1.3 |
| Dill | *Anethum graveolens* L. | 2 | 0.9 |
| Asparagus | *Asparagus officinalis* L. | 1 | 0.4 |
| Chard | *Beta vulgaris* var.cicla L. | 6 | 2.6 |
| Borage | *Borago officinalis* L. | 1 | 0.4 |
| Cabbage | *Brassica oleracea* L. | 12 | 5.1 |
| Pepper | *Capsicum annuum* L. | 11 | 4.7 |
| Chickpea | *Cicer arietinum* L. | 7 | 3.0 |
| Endive | *Cichorium endivia* L. | 3 | 1.3 |
| Chicory | *Cichorium intybus* L. | 1 | 0.4 |
| Watermelon | *Citrullus lanatus* (Thunb.) Matsum. & Nakai | 1 | 0.4 |
| Melon | *Cucumis melo* L. | 15 | 6.4 |

| Common name | Scientific name | Accessions (n) | Frequency (%) |
|---|---|---|---|
| Cucumber | *Cucumis sativus* L. | 1 | 0.4 |
| Summer squash | *Cucurbita pepo* L. | 2 | 0.9 |
| Pumpkin | *Cucurbita* spp. | 19 | 8.1 |
| Lettuce | *Lactuca sativa* L. | 13 | 5.6 |
| Grass pea | *Lathyrus sativus* L. | 5 | 2.1 |
| Parsley | *Petroselinum crispum* L. | 1 | 0.4 |
| Common bean | *Phasoleus vulgaris* L. | 38 | 16.2 |
| Pea | *Pisum sativum* L. | 29 | 12.4 |
| Common golden thistle | *Scolymus hispanicus* L. | 1 | 0.4 |
| Tomato | *Solanum lycopersicum* L. | 41 | 17.5 |
| Spinach | *Spinacia oleracea* L. | 4 | 1.7 |
| Broad bean | *Vicia faba* L. | 5 | 2.1 |
| Maize | *Zea mays* L. | 12 | 5.1 |
| | TOTAL | 234 | 100.00 |

Accessions from some species were found mainly in a specific area, signaling the adaptation of these species to the site's agroclimatic conditions and traditions. For instance, chickpeas (*Cicer arietinum* L.) were found mainly in ES5110015 (5 of the 7 accessions collected). In this area, the landrace "cigronet menut" (*small chickpea*) is highly valued by farmers and consumers. On the other hand, melons (*Cucumis melo* L.) were found mainly in ES5140011 (13 of the 15 accessions collected). This area historically produced melons for markets in the cities of Barcelona and Tarragona. Interviews with farmers suggest that no single melon variety is especially valued over others in this area; each accession has a different name, some of which suggest specific organoleptic traits (e.g., "meló de pell fina" (*thin-skinned melon*), "meló d'aroma" (*aromatic melon*)).

## Comparison of the Germplasm Collected in the New Missions with Germplasm Preserved *Ex Situ*

Comparison of data from *ex situ* collections with data obtained in our new collection missions reveals that a high diversity of CGR is still preserved on farm and some of this CGR was probably not captured by previously collecting expeditions. The indicators of species richness (mean values, $S_{new\ mission}$ = 12.8,

$S_{previous\ missions}$ = 7.0), variety richness ($V_{new\ mission}$ = 27, $V_{previous\ missions}$ = 11), and total abundance ($A_{new\ mission}$ = 53.8, $A_{previous\ missions}$ = 18.3) were significantly higher in the newly collected materials than in the *ex situ* database (Table 4). There were no significant differences in the Shannon-Weaver index (H1) between *ex situ* and new collections when considering species and accessions within species ($p = 0.1367$, Table 4), mainly because prior field surveys in ES5130029 collected a high number of species ($n = 5$) in relation to the total number of accessions ($n = 8$). Nevertheless, when considering the variety factor, a significant difference (at $p < 0.10$ level) was identified both for H2 (species and variety levels) and for H3 (variety and accession levels). Thus, high variability not yet reflected in seed banks is still present on farm, and this variability is found at both inter- and intra-variety levels.

**Table 4.**

Comparison of existing *ex situ* CGR (previous missions) and newly collected accessions natura 2000 areas. At the bottom, mean values and significance from the anova analysi

| Area of study | Previous missions/new mission | Species richness (S) | Variety richness (V) | Total abundance (A) | Shannon-Weaver i | | |
|---|---|---|---|---|---|---|---|
| | | | | | Species level ($H_1$)[a] | Species level ($H_2$)[b] | V |
| ES5130029 | Previous | 5 | 5 | 8 | 1.56 | 1.609 | 1. |
| ES5130029 | New | 8 | 14 | 40 | 1.50 | 1.909 | 1. |
| ES5110015 | Previous | 9 | 12 | 18 | 1.90 | 1.979 | 2. |
| ES5110015 | New | 17 | 39 | 80 | 2.33 | 2.415 | 3. |
| ES5140019 | Previous | 2 | 2 | 3 | 0.64 | 0.693 | 0. |
| ES5140019 | New | 8 | 16 | 26 | 1.74 | 1.906 | 2. |
| ES5140011 | Previous | 12 | 25 | 44 | 2.10 | 2.422 | 2. |
| ES5140011 | New | 18 | 39 | 69 | 2.55 | 2.684 | 3. |
| Mean values | Previous | 7.0 | 11 | 18.3 | 1.55 | 1.676 | 1. |
| | New | 12.8 | 27 | 53.8 | 2.03 | 2.228 | 2. |
| *p* value | | 0.0125 | 0.0254 | 0.0294 | 0.1367 | 0.0897 | 0. |

[a]$H_1$ = considering species and accession levels

| |
|---|
| [b]$H_2$ = considering species and variety levels |
| [c]$H_3$ = considering variety and accession levels |

Finally, we selected the most important landraces (i.e., those with a strong presence in markets and cuisine) to compare the number of accessions found in the new field survey with the number previously stored in seed banks (Table 5). Research groups' previous efforts to collect intra-varietal genetic variability for some landraces have had an important impact. For instance, "Ganxet" bean and "Penjar" tomatoes were highly represented in germplasm banks (388 and 361 accessions, respectively), and the number of newly collected accessions (10 and 11, respectively) was similar to those previously collected in the same areas (6 and 13, respectively). Nevertheless, some important landraces were not well represented in the gene banks; for instance, few accessions of "col brotonera" (*sprouting broccoli*) (*Brassica oleracea* L.), "blat de moro d'escairar" (*Zea mays* L.), "cigronet de l'Anoia" (*Cicer arietinum* L.), and "de la Creu," "del Benach," and "Poma/Pometa" tomato landraces were present in *ex situ* collections, so the newly collected materials will help represent the intravarietal genetic diversity of these landraces considerably.

**Table 5.**

A comparison of the *ex situ* accessions in seed banks and the accessions collected in the recent campaign in four natura 2000 areas for a group of selected landraces.

| Local name | Scientific name | New collecting mission | Previously collected accessions |
|---|---|---|---|
| Blat de moro d'escairar | *Zea mays* L. | 4 | 2 |
| Cigronet de l'Anoia | *Cicer arietinum* L. | 4 | 1 |
| Col brotonera | *Brassica oleracea* L. | 8 | 0 |
| Enciam negre | *Lactuca sativa* L. | 3 | 0 |
| Escarola cabell d'àngel | *Cichorium endivia* L. | 3 | 0 |
| Mongeta del Ganxet | *Phaseolus vulgaris* L. | 10 | 6 |
| Pèsol negre | *Pisum sativum* L. | 21 | 4 |
| Tomàquet de Penjar | *Solanum lycopersicum* L. | 11 | 13 |

| Local name | Scientific name | New collecting mission | Previously collected accessions |
|---|---|---|---|
| Tomàquet del Benach | *Solanum lycopersicum* L. | 2 | 0 |
| Tomàquet de la Creu | *Solanum lycopersicum* L. | 2 | 2 |
| Tomàquet Poma/Pometa | *Solanum lycopersicum* L. | 2 | 1 |

# Discussion

Increasing social awareness about genetic erosion of local landraces has led to various initiatives at national, regional, and local levels to collect and conserve seeds of these varieties. International research centers (mainly in the 1970s and 1980s) (Thormann et al. 2012) and nonprofit organizations and universities (mainly after 2000) have stored hundreds of accessions in seed banks. So, it is necessary to rationalize and prioritize further efforts devoted to collecting and conserving seeds of landraces/old varieties.

In Catalonia, presented here as a case study, of the total of 3568 accessions filed in seed banks, 2931 are unique accessions. Most of these (2121 accessions) are managed by nonprofit organizations or universities without regulated systems to maintain seeds in the long term. Importantly, only 21.7% of these accessions are present as duplicates in the National Inventory, making the long-term availability of these seeds uncertain. Furthermore, some landraces are overrepresented in the *ex situ* collections because breeding programs developed by Polytechnic University of Catalonia-Miquel Agustí Foundation have extensively collected seeds of “Ganxet” (388 accessions) (Casañas et al. 1997; Casañas et al. 1999) and “Santa Pau” beans (48 accessions) (Almirall et al. 2010), and of “Penjar” (361 accessions) (Casals et al. 2012), “Montserrat” (96 accessions) (Casals et al. 2011), and “Pera de Girona” tomatoes (61 accessions) (Casals et al. 2010). Given the high costs of *ex situ* conservation (Li and Pritchard 2009), studies are underway to characterize the genetic variability within these varieties at the agricultural, morphological, organoleptic, chemical, and DNA levels to build core collections of these varieties (i.e., a limited set (5–20% of the total collection) of accessions chosen to represent the genetic spectrum in the whole collection (van Hintum et al. 2000)). Characterization will make space in seed banks by reducing the number of accessions conserved *ex situ* without losing genetic variability, as has already been done for example with Spanish common bean genetic resources (Pérez-Vega et al. 2009; Rivera et al. 2016).

Our results show that the geographical coverage of the seed banks is low as only 35.3% of the municipalities in Catalonia are represented. Considering the high agroclimatic variability in Catalonia and the relationship between ecogeographical factors and patterns of plant genetic variation (Peeters et al. 1990; Russell et al. 2016), the low geographical coverage of seed banks is an important shortcoming in CGR conservation in this area. This irregularity is due to various factors, including the different interests of the institutions involved in CGR conservation, the location of the seed banks, and the lack of an integral plan for the conservation and use of CGR in Catalonia. GIS technology is increasingly used in germplasm collection missions (Jarvis et al. 2005), and GIS data developed in this study can be used to guide new collection missions.

Tomatoes and common beans were the species with the most accessions collected in our field surveys and are also the species with the most accessions in the *ex situ* collections. These results are not surprising, as tomatoes and beans are the species most frequently conserved by farmers in the Mediterranean basin (Montesano et al. 2012; Thomas et al. 2012). Farmers value landraces from these species for different reasons. Tomato landraces' organoleptic profile is considered superior to that of modern varieties (Casals et al. 2011; Sinesio et al. 2007), and the "Penjar" landrace is valued in Catalonia for its long shelf life (Casals et al. 2012). Common beans have historically been appreciated for their nutritive properties and for their ability to be stored, and they are key ingredients in many traditional Catalan dishes.

One of the main shortcomings of the *ex situ* conservation system in Catalonia is that the diversity of species and varieties are not well represented in the *ex situ* collections. Our results in the model areas show that diversity calculated at species (species richness (S) and Shannon-Weaver index (H2)) and variety levels (variety richness (V) and Shannon-Weaver index (H3)) are significantly higher on farm than *ex situ* (Table 4). Thus, the representation of the diversity (at species, variety, and intra-varietal levels) in *ex situ* collections does not accurately reflect what is still cultivated on farm. These differences are probably due to incomplete collecting activities in previous missions. In the present project, we spent 3 days collecting seeds in each municipality. This highly intensive scheme enabled us to capture nearly all genetic resources grown on farm in these areas, making it possible for us to compare *ex situ* (what is filed in seed banks) with in situ (what we have collected) collections. Nevertheless, as the collection missions took place at different times, a fraction of the differences between *ex situ* and in situ may be due to the introduction of new varieties through immigration or crosses and selection (although an unknown number have also been lost in the meanwhile). More importantly, for some economically relevant landraces, the number of accessions conserved *ex situ* is very low (Table 5). Apart from genetic erosion issues, this can be an important gap for farmers interested in cultivating these varieties for market-driven productions in the future. These results show that further efforts to capture the genetic variability among vegetable CGR still cultivated, if planned with stricter methods and carried out with greater efficiency, can yield valuable results. Furthermore, our analysis underlines the need to obtain phenotypic or molecular data of accessions conserved *ex situ* in order to identify possible homonymies (i.e., accessions with similar varietal names but with different genetic fingerprints) and synonymies (i.e., duplicates).

The genetic erosion rate in the last century has been high in all crops (Hammer and Teklu 2008; van de Wouw et al. 2010) and is a major concern for agriculture. For instance, the genetic erosion in cereals (excluding maize) has been estimated from 80 to 100% (Hammer et al. 1996; Hammer and Laghetti 2005). Although our study did not specifically target cereals, our mission collected only two accessions of wheat (*Triticum aestivum* L.) and no accessions of other cereals important in our area such as barley (*Hordeum vulgare* L.) or oats (*Avena sativa* L.). By contrast, our results show that although landraces of vegetables and pulses have been replaced by modern varieties (nearly 100%) in market-driven productions, the replacement rate in vegetable gardens is much lower and genetic diversity has been preserved (and probably created de novo). In fact, only one landrace mentioned by farmers could not be found during our missions; this landrace ("safranòria," a red carrot previously cultivated in ES5140011 area) was also absent from the *ex situ* collections and can be considered extinct. However, farmers still conserve seeds of the remaining landraces with at least some recognizable traits.

The present study shows that although germplasm banks conserve significant genetic variability, the real genetic diversity found in situ is even greater and could increase the genetic diversity held *ex situ*. So, we propose the following actions to increase the efficiency of the CGR conservation system:

(a) A single institution should be in charge of analyzing and coordinating publicly funded CGR network activities. This institution should guide future prospection missions to unexplored areas and/or focus on specific species/varieties that are poorly represented in *ex situ* collections.

(b) All the CGR collected with public funding should be stored as duplicates in the National Inventory. A clear material transfer agreement (MTA) should be reached with farmers in order to protect their rights on possible agricultural innovations developed from these materials.

(c) Universities and botanical gardens should take advantage of their collecting activities to also collect species that are not of particular interest for their plant breeding or conservation programs.

(d) A coordinated system of phenotyping using consensus descriptors should be created to document the agricultural and morphological characteristics of stored accessions to enhance their use.

(e) Phenotypic and genotypic data should be used to detect duplicates and to develop core collections to reduce the number of accessions that need to be stored.

In summary, our results show that better organization and coordination between major and minor actors involved in the CGR conservation network are needed to increase the efficiency and coverage of the collections and the study of established and newly emerging landraces.

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