

# Current Issues in Cereal Crop Biodiversity

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**Abstract** The exploration, conservation, and use of agricultural biodiversity are essential components of efficient transdisciplinary research for a sustainable agriculture and food sector. Most recent advances on plant biotechnology and crop genomics must be complemented with a holistic management of plant genetic resources. Plant breeding programs aimed at improving agricultural productivity and food security can benefit from the systematic exploitation and conservation of genetic diversity to meet the demands of a growing population facing climate change. The genetic diversity of staple small grains, including rice, maize, wheat, millets, and more recently quinoa, have been surveyed to encourage utilization and prioritization of areas for germplasm conservation. Geographic information system technologies and spatial analysis are now being used as powerful tools to elucidate genetic and ecological patterns in the distribution of cultivated and wild species to establish coherent programs for the management of plant genetic resources for food and agriculture.

**Keywords** Biotechnology · Climate change · Crop improvement · Food security · Genetic resources

## List of Abbreviations

|       |   |
|-------|---|
| AVRDC | The World Vegetable Center                                |
| BNI   | Biological Nitrification Inhibition                       |
| CIAT  | International Center for Tropical Agriculture             |
| CGIAR | Consultative Group on International Agricultural Research |
| CGRP  | Canadian Genetic Resources Programme                      |

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| DNA      | Deoxyribonucleic acid  |
| FAO      | Food and Agriculture Organization of the United Nations                  |
| GBIF     | Global Biodiversity Information Facility                                 |
| GCDT     | Global Crop Diversity Trust  |
| GEF      | Global Environment Facility  |
| GHG      | Greenhouse gas   |
| GM       | Genetically modified   |
| GMO      | Genetically modified organism  |
| IBPGR    | International Board for Plant Genetic Resources                          |
| ICRISAT  | International Crops Research Institute for the Semi-Arid Tropics         |
| IFAD     | International Fund for Agricultural Development                          |
| ILRI     | International Livestock Research Institute                               |
| IRD      | Institut de Recherche pour le Développement                              |
| ITPGRFA  | International Treaty on Plant Genetic Resources for Food and Agriculture |
| IUCN     | International Union for Conservation of Nature                           |
| JIRCAS   | Japan International Research Center for Agricultural Sciences            |
| MLS      | Multilateral system  |
| NBPGR    | National Bureau of Plant Genetic Resources (India)                       |
| NUS      | Neglected and underutilized species                                      |
| ORSTOM   | Office de la Recherche Scientifique et Technique d'Outre-Mer             |
| SINGER   | System-wide Information Network for Genetic Resources                    |
| SMTA     | Standard Material Transfer Agreement                                     |
| T-DNA    | Transfer-deoxyribonucleic acid   |
| TALENs   | Transcription activator-like effector nucleases                          |
| UNEP     | United Nations Environment Programme                                     |
| UNU      | United Nations University  |
| USDA-ARS | United States Department of Agriculture, Agricultural Research Service   |
| WEMA     | Water Efficient Maize for Africa   |
| WHO      | World Health Organization  |

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## 1 Introduction

Agricultural biodiversity is the key to successful biotechnological approaches for crop improvement. Plant breeding programs aimed at increasing crop productivity and improving food security rely on traits that must be efficiently managed and exploited for the sustainable delivery of cultivars without compromising genetic diversity. Exploration of new biodiversity in the face of climate change and the threat of genetic erosion must complement current trends in crop diversity. Enhanced conservation strategies of important agricultural biodiversity need to be deployed to take advantage of cutting-edge technologies on spacial analysis to monitor the patterns of plant diversity and distribution and prioritize areas for conservation. Most recent advances in the genetic modification of crop plants (i.e. transgenics) are also an important issue for agricultural production. Next-generation crops need to be created using transdisciplinary strategies, with the goal of making farming more productive and more profitable. Major staple crops, such as rice, maize, wheat, millets, and most recently quinoa, play an important role for millions of people worldwide because these crops sustain the lives of the poorest rural farmers. Therefore, diversity and conservation issues involving the utilization of these staple crops will significantly contribute to a household’s food security and nutrition of smallholders, mainly in the tropics.

## 2 Agricultural Biodiversity: Concept, Importance and Scope

Agricultural biodiversity, also known as agrobiodiversity, can be defined as all of the components of biological diversity that are relevant to food and agriculture, including agricultural ecosystems [1]. From a pragmatic perspective, agrobiodiversity is the result of the interaction between the environment, genetic resources, and management systems and the practices used by people from diverse cultural backgrounds.

Agrobiodiversity is an integral part of overall biodiversity; it comprises the variety and variability of animals, plants, and microorganisms at the genetic, species, and ecosystem levels that are used for food and agriculture, including crops, livestock, forestry, and fisheries. Culture and local knowledge are regarded as essential parts of agrobiodiversity because it is the human activity of agriculture that affects and shapes this biodiversity. In other words, agrobiodiversity is the result of natural selection and human intervention over millennia, and it plays a key role in sustainable development, including processes for, and in support of,

food production and food security [2, 3]. Some part of this biodiversity is directly managed to supply the goods and services that people need; however, most of it is not directly intended for production purposes and remains important as a source of materials for its contributions to ecosystem services, such as pollination, control of greenhouse gas emissions, and soil dynamics [4].

The diversity in crops consists of the crops, landraces, and cultivars grown by farmers [5]. The world is currently facing serious environmental problems due to loss of biological diversity at alarming rates. Scientists have estimated that by 2025, 60,000 plant species could be lost. The Food and Agriculture Organization of the United Nations (FAO) has also stated that, since 1900, approximately three quarters of the genetic diversity of domestic agricultural crops has already been lost [6].

It is well known that modern, intensive agriculture reduces agricultural biodiversity [4, 5]; this loss of genetic diversity in agriculture is known as genetic erosion [7]. It is commonly assumed that plant breeding efforts are an important cause of genetic erosion of crops. However, the effects of urbanization and modern agricultural practices are important factors as well. Climate change and environmental degradation can also contribute to changes in cropping patterns and the disappearance of traditional varieties [5].

The spread and adoption of modern crop varieties has implications for genetic erosion and a decline in crop genetic diversity. Conservation of crop genetic resources is therefore a prerequisite for future generations. Organized and well-focused exploitation and conservation strategies of biodiversity will allow users to breed crop varieties for improved food security and face new challenges in the era of climate change.

## ***2.1 Conservation of Agrobiodiversity in Small Grains: Status and Applications***

It is generally accepted that the modernization of agriculture and land use changes negatively affect economic plant diversity, both on farmers' fields and in home gardens [5]. This might eventually lead to the genetic erosion of cultivars and crop wild relatives with potentially useful traits for current and future human use. Therefore, many collecting missions have been organized in the past decades to establish extensive international and national genebank collections for important food crops, including cereals [8]. More than 3 million cereal accessions (i.e. samples of living plant material collected from particular locations) are stored *ex situ* worldwide [9]. They account for almost half of all genetic materials conserved globally in genebank collections [9]. This confirms the importance of cereals for global food security and agricultural production. The three crops with the most accessions conserved *ex situ* at a global level are the cereals rice (*Oryza* spp.), wheat (*Triticum* spp.), and barley (*Hordeum vulgare* subsp. *vulgare*) [9]. Of other cereal crops, such as maize (*Zea mays* L.), sorghum (*Sorghum* spp.), oat (*Avena sativa* L.), and millets (e.g. *Pennisetum glaucum*), less but still huge amounts of

materials are being conserved *ex situ* [9]. To facilitate access to genetic material for evaluation, breeding, and direct use, some of these collections are put in the Multilateral System (MLS) under the conditions defined in the International Treaty on Plant Genetic Resources for Food and Agriculture ([www.planttreaty.org](http://www.planttreaty.org)). Several of the collections that are in the open domain, such as those held in trust by the Consultative Group on International Agricultural Research (CGIAR), can be consulted through the GENESYS Web portal (<http://www.genesys-pgr.org>), developed by Bioversity International.

The genetic integrity of accessions is maintained as much as possible in *ex situ* gene bank collections to conserve the specific characteristics of each material for evaluation, breeding, and direct use. For example, most accessions of the cereal collections held in trust by CGIAR have been characterized (88 %) [9]. This percentage is higher than all other types of crops conserved by CGIAR centers and The World Vegetable Center [9]. However, considering all reported national collections around the world, the amount of characterized cereal genetic resources is lower and similar to levels of genetic resource characterization of other crop types [9]. As a drawback, *ex situ* collections do not maintain the continued process of interactions between plants, humans, and environmental factors that take place on farms and between farmers [10]. *In situ* conservation of crops such as cereals is thought to be important to maintain the adaptive genetic variation of crop populations through interactions with their environment, including human management and selection. Several newly sampled barley materials in Morocco, for example, had more disease resistance than accessions collected several decades ago at the same location [11]. This supports the need of cereal genetic resources in situ conservation to allow evolution of adaptive genetic variation to important diseases for overall crop production. On the other hand, some historic *ex situ* accessions included rare genes to resistance that were extinct in the current species populations due to genetic erosion [11]. This highlights the importance of *ex situ* conservation as well as the necessity to develop complementary strategies of *ex situ* and in situ conservation.

Therefore, there is a need to assess the diversity status and dynamics of in situ plant genetic resources and develop complementary *ex situ* and in situ conservation strategies [9, 12–14]. At the same time, these types of analyses are useful to identify remaining gaps of diversity that are missing in existing genebank collections and that should therefore be targeted for germplasm collecting [9, 13]. Of course, periodic monitoring activities are required to measure the effectiveness of in situ conservation over time and to check the viability of seed material in *ex situ* collections.

The main purpose of in situ conservation is to maintain genetic variation in cultivated crop populations for phenotypic selection by farmers and/or natural processes [15]. This allows maintenance of the processes of microevolution and continuous adaptation of crop populations to their environments, including farmer management. The genetic structure of populations can change when phenotypic traits are heritable and selection is sufficiently strong. Following Darwin's concepts of selection, this allows cumulative directional genetic response over generations—that is, microevolution of these populations to natural and human selection [15, 16]. Microevolution in plant populations is further driven by factors

such as random mutation, recombination, and genetic drift [17, 18]. As an additional factor, many smallholders in all parts of the world periodically introduce new materials from neighbors and other localities into their systems to sustain productivity [19]. These factors and activities together make on-farm plant genetic resources management a dynamic system of crop genetic diversity use. Farmers may select for changing preferences as well choose to maintain desired phenotypic traits [15]. The variety of traits that is maintained and evolving under farmers' care is often unknown to conventional breeders, entrepreneurs, and consumers. This makes on-farm conservation areas potential sources of untapped diversity for the development of new crop varieties for local and wider use. Even genetic diversity itself in cultivated populations may be a trait of farmers' selection for ecosystem services, such as pest and disease control [20].

The status and trends of intraspecific crop diversity are traditionally being assessed and monitored through varietal diversity, either through farmer interviews or morphological or botanical classifications. For example, classification on the basis of traits that are important for farmers suggests that sorghum and pearl millet varietal diversity in Niger remained at similar levels during the last three decades of the twentieth century [21]. A reason for the maintenance of varietal diversity could be that the areas under study are marginal terrains where the cultivation of traditional crop and varieties outperforms cash crops and/or crop genetic diversity is being maintained as a risk management strategy [21]. Taxonomic keys have been used to monitor traditional maize varieties ([www.biodiversidad.gob.mx/genes/proyectoMaices.html](http://www.biodiversidad.gob.mx/genes/proyectoMaices.html)). Taxonomic studies are the basis for understanding variation in plant genetic resources. However, assessment of varietal diversity according to morphological or botanical characterization may still lead to a substantial degree of misidentification [22], and taxonomic keys may not exist for botanical varieties of specific crops or from particular geographic areas. This limits intraspecific diversity studies.

Molecular tools that identify polymorphisms have created novel opportunities for assessing crop genetic diversity, particularly when these markers are linked to adaptive traits and applied in combination with new geospatial methods of geographic and environmental analysis [23–25]. In addition, geographic information systems (GIS) can contribute significantly to improving the understanding and monitoring of spatial and temporal patterns of crop diversity [26]. Application of spatial analyses on georeferenced diversity data allows the formulation and implementation of better-targeted, and hence more effective, conservation strategies of inter and intra-specific plant diversity. For example, geospatial analyses combined with molecular marker characterization data have been used to support conservation strategies for African rice (*O. glaberrima* Steud.) genetic resources [27]. This study found the highest African rice genetic diversity in intermediate humid conditions and a decrease of genetic diversity under semiarid conditions and humid conditions [27]. This may have implications for the genetic resource conservation of this crop under changing climate conditions [27].

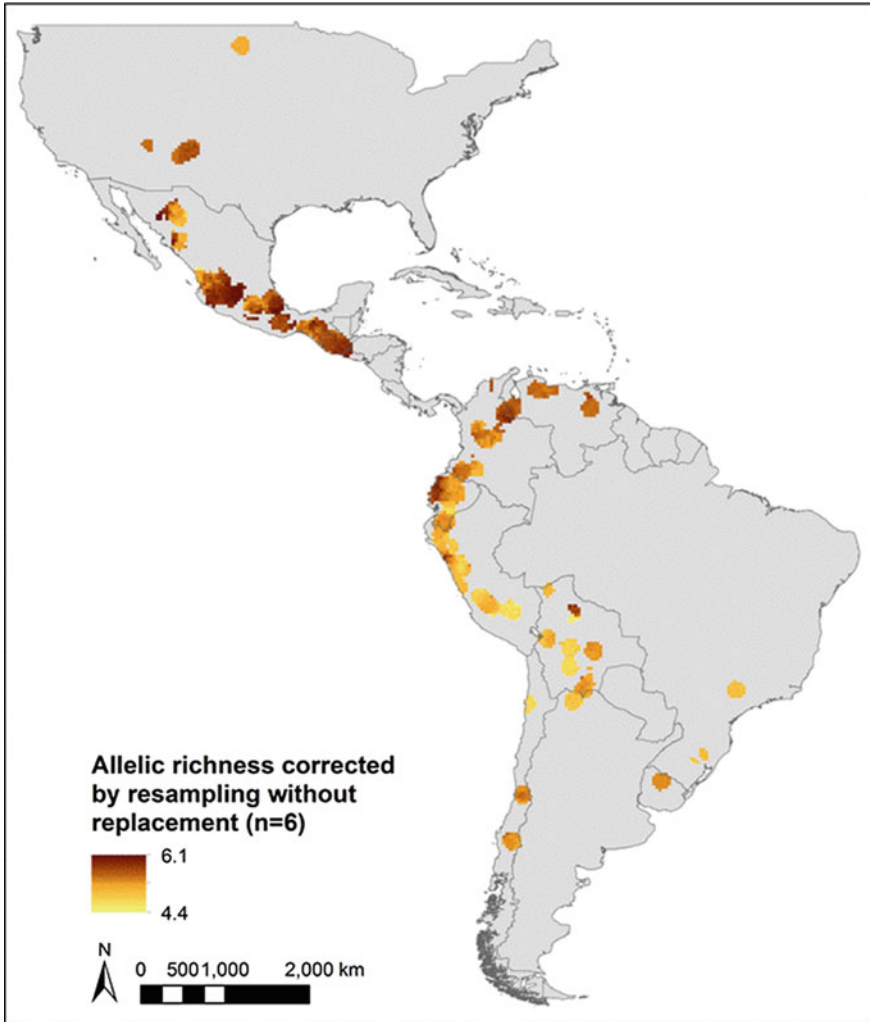
As an example of how to apply geospatial analysis to support plant genetic resources conservation, maize microsatellite diversity was mapped in the Americas. A dataset freely available by the Genetic Architecture of Maize and Teosinte

project at [www.panzea.org](http://www.panzea.org) was used. It consists of molecularly characterized maize accessions from different genebanks. This dataset has been used by Vigouroux et al. [22] to understand the genetic structure (*beta* diversity) in America's maize distribution. Four big genetic groups can be distinguished, consisting of material from (1) temperate zones in the United States and South America; (2) Mexican highlands; (3) tropical lowland; and (4) the Andes [22]. This data was used to map maize *alpha* diversity. A total of 1,145 georeferenced accessions that had micro-satellite data for 92 markers were selected. Following van Zonneveld et al. [28], a 20 min grid layer (corresponding to approximately 33 km in the study area) was constructed for all genetic parameters, applying a circular neighborhood with a diameter of two degrees (corresponding to approximately 222 km) to improve visualization and group geographically isolated germplasm accessions. Calculations were done in the R program version 2.15.1 with the packages Raster [29] and Adegenet [30]. To standardize comparability of these parameters between cells, sample bias was corrected through resampling without replacement after Leberg [31] to a sample size of six trees. Per parameter, the average value was calculated from six subsamples following the bootstrap method developed by Thomas et al. [32].

The highest allelic richness is found in the central highlands of Mexico (Fig. 1). This indicates a high variation of maize genetic resources, and this area therefore should be prioritized for conservation actions. Nevertheless, the center of maize domestication is thought to be located in the southern lowlands, which thus is also an important area for conservation [33]. Also, a high number of alleles is found in these areas (Fig. 1). In a few scattered areas in northern Mexico, high levels of diversity are also observed.

Allelic richness is lower in South America, where maize was introduced later. However, high levels of diversity can be found in Ecuador in the Andean region, Venezuela, northern Colombia, and Bolivia in the tropical lowlands (Fig. 1). These materials belong to different genetic groups than the material from the Mexican highlands [22]. These clusters may reflect different groups of evolution and adaptation to different environments. To maximize conservation of plant genetic resources, areas from these clusters that harbor high genetic variation should be prioritized for conservation.

The high genetic maize diversity in the Mexican highlands can be explained by the high levels of introgression between crop wild relatives and maize in that area [33, 34]. Indeed, gene flow between cultivated plants and their relatives in overlapping areas of distribution can cause elevated levels of intraspecific cultivated plant diversity. Such insights allow a better understanding of the role of evolutionary processes in the development of current species distributions and, where relevant, their domestication [35]. Similar phenomena have also been observed in other areas. Higher levels of molecular diversity of domesticated emmer wheat (*Triticum turgidum* L. subsp. *dicoccon* (Schrank) Thell.) and bread wheat (*T. aestivum* L.) have been found in the eastern Mediterranean and Turkey, respectively, and are located south and west of their putative centers of domestication due to crossing between domesticates and their wild ancestors [36].



**Fig. 1** Maize microsatellite diversity in the Americas constructed from geospatial analysis as a tool for genetic resources conservation. The average number of alleles per locus is shown

In addition to studies across crop distribution ranges to target areas for in situ conservation and germplasm collecting, local spatial studies in prioritized traditional rural communities are important to provide input to the development of appropriate on-farm PGR management strategies [25]. These studies help to (1) increase the understanding of how farmers manage and conserve crop diversity within a community and; (2) identify the optimal geographic scale for interventions and crop diversity monitoring, as well as the social context in which in situ conservation should be implemented [37]. Estimates of distribution and levels of crop diversity in rural communities also help to determine the need to introduce



new varieties into local seed systems and improve seed distribution systems accordingly [38].

Several case studies illustrate how genetic analysis helps to support in situ conservation interventions. A microsatellite marker study of diversity and structure of local rice varieties (*Oryza sativa* L., *O. glaberrima* Steud.) in the Republic of Guinea revealed genetic differences between two different agro-ecosystems, but no differentiation was shown between villages or farms within each of the contrasting agro-ecosystems [37]. This suggests that most genetic diversity can be conserved within just a few farms of a village [37]. Simple sequence repeat (SSR) markers also detected high seed exchange within villages of a traditional rice variety in Thailand [39]. In addition, high genetic differentiation between villages was found [39]. This indicates long periods of local adaptation and selection. Also, low genetic differentiation between seed lots of different farmers of the specific Mexican maize variety, Jala, suggests high seed exchange between farmers [40]. This implies that within communities only a few farmer fields would be necessary to target for in situ conservation and that collection for *ex situ* conservation of many individuals in a few farmer fields is preferred to collection of a limited number of individuals in many fields [40].

Climate change will certainly impact landraces conserved in situ, such as the native maize races in Mexico [18]. Nevertheless, it remains difficult to predict whether a local landrace goes extinct or can adapt [18]. This depends partly on the magnitude of the climate alterations, the novelty of new climates, and the amount of genetic variation present in landrace populations [18]. Seed exchange between farmers remains an important means to adapt local seed material to changing climates. Ecogeographic analysis of traditional maize systems in Mexico shows that mid-elevation communities can adapt fairly easy their production systems to climate change through seed exchange with farmers within a 10 km radius, where a wide range of different micro-climates can be found [41]. In contrast, highland and lowland systems that have less local micro-climate diversity require seed material from geographically more distant locations. The latter would require active support from governmental and non-governmental organizations [41].

During the domestication process of crops, overall genetic diversity is generally reduced, whereas phenotypic diversity at specific parts of the genome related to traits of interest are increased. However, many unknown traits of interest may have been lost during that domestication process. Crop wild relatives and progenitors may still include many potentially interesting traits, such as adaptive traits to heat stress and other climate change-related traits [42]. Therefore, recently more emphasis has been placed on the conservation of crop wild relatives. Microsatellite studies on rice in Vietnam confirm that wild rice populations contain much more diversity than cultivated populations [43]. A good example is mapping the genetic diversity of the wild subspecies of barley (*Hordeum vulgare* subsp. *spontaneum*) in Eastern Mediterranean and Middle East (Israel, Palestine, Syria, and Turkey), and Central Asia [44]. In these locations, barley here has higher genetic diversity in the eastern Mediterranean than elsewhere in its distribution. This area should therefore be a focus of conservation activities for barley genetic resources.

## **2.2 Genetic Diversity of Pearl Millet and Its Wild Relatives: Distribution, Conservation, and Use**

### **2.2.1 Background**

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is the fifth most important cereal crop in the world after rice, wheat, maize, and sorghum. It is a widely grown rainfed cereal crop in the arid and semi-arid regions of Africa and Southern Asia, and it can be grown in areas where rainfall is not sufficient (200 to 600 mm/year) for the cultivation of maize and sorghum. In other countries, it is grown under intensive cultivation as a forage crop. Pearl millet accounts for almost half of global millet production, with 60 % of the cultivation areas in Africa, followed by 35 % in Asian countries. European countries represent 4 % of millet cultivation and North America only 1 %, mainly for forage. Today, millet is a staple for more than 500 million people. Areas planted with pearl millet are estimated at 15 million ha annually in Africa and 14 million ha in Asia. Global production exceeds 10 million tons a year [45]. In sub-Saharan Africa, pearl millet is the third major crop, with the major producing countries being Nigeria, Niger, Burkina Faso, Chad, Mali, Mauritania, and Senegal in the West and Sudan and Uganda in the East. In Southern Africa, maize has partially or completely displaced millet cultivation because of commercial farming. India is the largest producer of pearl millet, both in terms of area (9.3 m ha) and production (9.3 mt), with an average productivity of 1044 kg/ha during the last 5 years (2007–2011).

The trends in area, production, and productivity of pearl millet suggest that area has increased marginally (2 %) during the last 2 years (2010–2011) and productivity has gone up by 19 % [46]. The major pearl millet growing states in India are Rajasthan, Maharashtra, Gujarat, Uttar Pradesh, Haryana, Karnataka, Madhya Pradesh, Tamil Nadu and Andhra Pradesh. However, productivity is the highest in Haryana, followed by Gujarat, Uttar Pradesh, Madhya Pradesh, Tamil Nadu, Andhra Pradesh, Rajasthan, Maharashtra, and Karnataka. It is mainly cultivated during the *Kharif* (rainy) season across the country. However, it is also grown to a lesser extent during the *Rabi* (post-rainy) season in Andhra Pradesh, producing high yields and excellent grain quality. Outside Africa and India, millets are also grown in Australia, China, Canada, Mexico, Russia, and the United States, primarily grown as a forage crop for livestock production [45]. Pearl millet is endowed with enormous genetic variability for various morphological traits, yield components, adaptation, and quality traits. Pearl millet is also nutritionally superior compared to maize and rice. The protein content of pearl millet is higher than maize and it has a relatively high vitamin A content.

### **2.2.2 Taxonomy, Origin, and Distribution**

Pearl millet, *Pennisetum glaucum*, is an annual, allogamous, cross-pollinated, diploid cereal belonging to the *Poaceae* family, subfamily *Panicoideae*, tribe

*Panicaceae*, subtribe *Panicinae*, section *Penicillaria*, and genus *Pennisetum*. The genus *Pennisetum* contains about 140 species. The important wild relatives of cultivated pearl millet include the progenitor, *Pennisetum glaucum* subsp. *monodii* Maire, *P. purpureum* K. Schumach, *P. pedicellatum* Trin., *P. orientale* Rich, *P. mezianum* Leeke, and *P. squamulatum* Fresen. Previous names are *P. typhoideum* L.C. Rich and *P. americanum* (L.) Leeke. The four cultivated forms of pearl millet are *typhoides* (found mainly in India and Africa), *nigritarum* (dominant in eastern Sahel), *globosum* (dominant in the western Sahel, probably originating in Sahelian Africa in a diffuse belt stretching from western Sudan to Senegal), and *leonis* (dominant on the West African coast) [47–49].

Linnaeus [50] originally placed pearl millet cultigens into two separate species (*P. glaucum* and *P. americanum*) of the genus *Panicum*. Later, he moved several of these elements to the genus *Holcus* [51]. Rechard [52] grouped pearl millet along with a number of species previously listed under both *Panicum* L. and *Cenchrus* L. in a new genus, *Pennisetum*. Willdenow [53], however, established the genus *Penicillaria* to include pearl millet, but Steudel [54] reduced it to its present status as a section in *Pennisetum*. He merged many variants of pearl millet into a single polymorphic species, recognized as *P. typhoideum* L. Rich. The limits of the section were expanded by Leeke [55] to include all those wild species of *Pennisetum* having penicillate anther tips and involucre bristles. The generic name *Pennisetum* has been derived from two Latin words, *Penna* and *Seta*, meaning feather and bristles (i.e., feathery bristles). The most extensive treatment of the genus *Pennisetum* was contributed by Stapf and Hubbard [56], who divided the genus into five sections: *Gymnothrix*, *Brevivalvula*, *Penicillaria*, *Heterostachya*, and *Eu-pennisetum*. Cultivated pearl millet and its wild and weedy relatives were included in section *Penicillaria*, which included 14 cultivated, 6 wild, and 13 intermediate species. Brunken [47] further reduced the number of species in section *Penicillaria* to two, on morphological and cytological grounds; *P. purpureum* was maintained as a separate species because of its tetraploid chromosome number and perennial lifecycle.

All the diploid cultivated, weedy, and wild taxa that frequently hybridize without genetic barriers are classified under a single species, *P. americanum*. Based on the morphology and adaptive strategies to domestication, *P. americanum* was further divided into three subspecies: *americanum*, including the cultivated forms; *monodii*, including the wild forms; and *stenostachyum*, with the weedy forms. Clayton and Renvoize [57] demonstrated that the taxonomically correct name for cultivated pearl millet is *P. glaucum*. They recognized the weedy forms (colloquially called *shibra*) as *P. sieberanum* and their wild progenitor as *P. violaceum*. *P. violaceum* differs from pearl millet in having involucre that are sessile, deciduous at maturity, and always contain a single spikelet.

The geographical origin and the center of domestication of pearl millet are situated in western Africa. The plant was subsequently introduced into India, where the earliest archaeological records date back to 2000 BC [48, 58–60]. Records exist for cultivation of pearl millet in the United States in the 1850s, and the crop was introduced into Brazil in the 1960s. The oldest findings of wild and

domesticated pearl millet were recorded at about 3500 BC in Dhar Tichitt, a Saharan site in Mauritania [61]. Birimi in northern Ghana has laid claim to one of the earliest findings of domesticated pearl millet, dated at about 1459 BC [62, 63].

These archaeobotanical findings in the Sahara and Sahel confirm the hypothesis of original distribution and widespread utilization of wild and cultivated pearl millet across sub-Saharan Africa [61, 62]. However, there is dispute among scholars as to whether pearl millet has a single center of origin or more than one place of origin (the so-called non-centers), which would have resulted from domestication processes occurring independently in several regions. According to the latter hypothesis, the whole Sahel, from Mauritania to western Sudan, was originally covered with these non-centers [60, 64–67]. Whether domestication took place as multiple parallel processes in the non-centers in several places along the Sahelian distribution belt of the wild progenitors or at one specific place [60, 68], the ultimate center of origin of the wild progenitors, *P. monodii* and *P. mollissimum*, is most likely to be situated in the Sahara desert [62, 63, 69].

Based on the distribution of pearl millet throughout the continent, the uniform cradle of domestication is likely to be the regions of Mauritania, Senegal, and western Mali [48, 68, 70]. Today's cultivated forms developed out of this domestication cradle [71]. Next, these first early-maturing forms of domesticated pearl millet were carried eastwards, facilitated by their efficient adaptation to arid conditions [62]. About 3,000 years BC, the first translocation carried the crop to eastern Africa [68, 71] and then to India, where 3000-year-old carbonized pearl millet was detected at a site on the eastern coast [63, 69].

Another diffusion took place in the region near Lake Chad, more precisely on the Nigerian side [72], where a secondary center of diversity developed at about 2100 BC [68, 73]. There, photoperiod-sensitive cultivars were selected, which adapted to the more humid conditions in the southern Sudanian zone [62, 71]. These late-maturing cultivars were transported further towards the Sudanian zone of southwestern Africa, from northern Nigeria to southern Senegal, as evidenced by the above-mentioned findings in northern Ghana [68, 73]. The third and last major translocation took pearl millet towards the plateaus of southern Africa, across Uganda and towards Namibia, at about 1000 BC [68, 71].

### 2.2.3 Overview of Pearl Millet Collections

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has the single largest consolidated collection of pearl millet in the world, which comprises a total of 22,211 accessions. Of these, 750 are of wild species (24 species), 19,377 are landraces, 132 are improved cultivars, 1,943 are breeding/research materials, and 25 are others [74]. India contributed a significant number of pearl millet accessions to the global collection maintained at ICRISAT (6,647 accessions). The remaining accessions were collected from about 51 countries. The major diversity centers of pearl millet are considered to be relatively well represented in the collection at ICRISAT. In addition to ICRISAT, the Institut de

Recherche pour le Développement in France also maintains 3,968 accessions of pearl millet from 16 countries. Collection of these accessions was supported by Bioversity International and Office de la Recherche Scientifique et Technique d'Outre-Mer. The Canadian Genetic Resources Programme in Saskatoon, Canada maintains 3,821 accessions covering a few species, with emphasis on *Pennisetum glaucum* (3390 accessions). Accessions of other species include: *P. violaceum* (221), *P. macrourum* (1), *P. purpureum* (12), *P. orientale* (1), *P. pedicellatum* (11), *P. polystachion* (8), *P. ramosum* (3), *P. unisetum* (1), and other species (14). The number of seeds maintained in these collections is moderate for long-term conservation; it is intended as safety duplication but not for distribution. In addition to these global collections, the Agricultural Research Station of the U.S. Department of Agriculture (USDA) at Griffin, Georgia maintains 1,314 accessions from 32 countries, of which only 1 is a wild relative, 290 are of breeding/research material, and 552 are for other purposes.

Among the national collections, the largest was recorded from the Indian genebank at the National Bureau of Plant Genetic Resources (NBPGR), which maintains 8,913 accessions under long-term conservation. Most of the accessions are indigenous (8,827), with only 168 accessions from other countries. The Indian collection also includes 221 advanced improved varieties and 272 accessions of breeding/research material. No other countries in South Asia, except Pakistan (193 accessions), have reported pearl millet collections. Among African countries, the collections were reported from gene banks based in Algeria, Benin, Botswana, Burkina Faso, Burundi, Cameroon, Central African Republic, Chad, Eritrea, Ethiopia, Ghana, Kenya, Malawi, Mali, Mauritania, Mozambique, Namibia, Niger, Nigeria, Senegal, Sierra Leone, Sudan, Democratic Republic of the Congo, Uganda, Zambia, and Zimbabwe. In total, 56,580 pearl millet accessions were recorded from various sources. Landraces represent the largest proportion of pearl millet germplasm conserved in gene banks worldwide (49,973 accessions). Of these, only 3 % are wild relatives collections (1,630), 0.80 % are advanced improved varieties (452), 6 % are breeding/research materials (3,600), and 2 % are of unknown description (947).

Some progress has been made in the recent past in mapping the pearl millet diversity collected worldwide. Global databases show that georeference data have been assigned to 16,855 accessions. These collections are being maintained at ICRISAT (13542 accessions), USDA Agricultural Research Service (ARS) (472 accessions) and the International Livestock Research Institute (13 accessions). With support from Bioversity International, 2,828 accessions were collected and became part of the global collections being maintained by ICRISAT and USDA-ARS. Not much information is available for any of the national collections. Based on the georeference information from the global database, the distribution pattern of these accessions is shown in Fig. 2, whereas the mapping of *Pennisetum* species (excluding *Pennisetum glaucum*) is shown in Fig. 3.

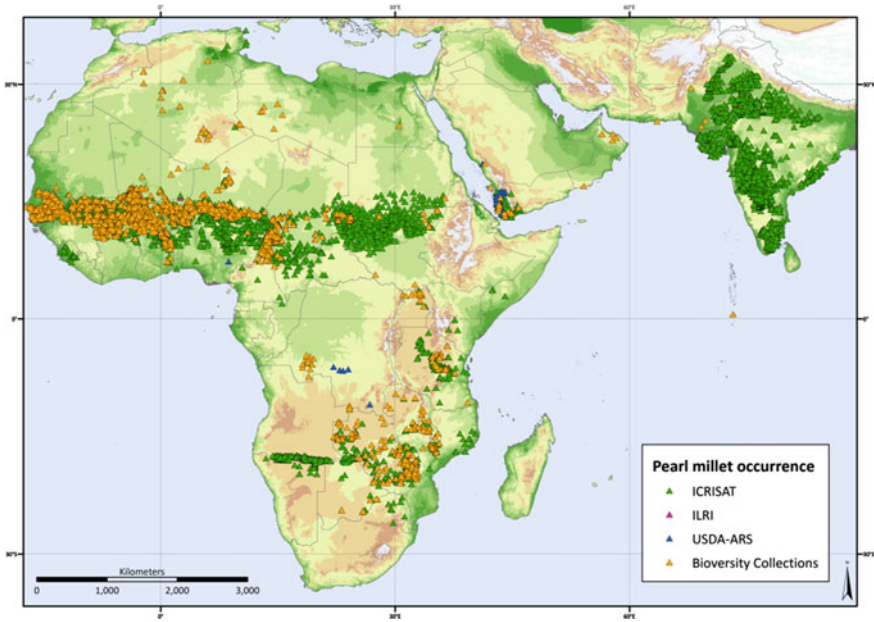


Fig. 2 Mapping of pearl millet accessions based on information available from the global database

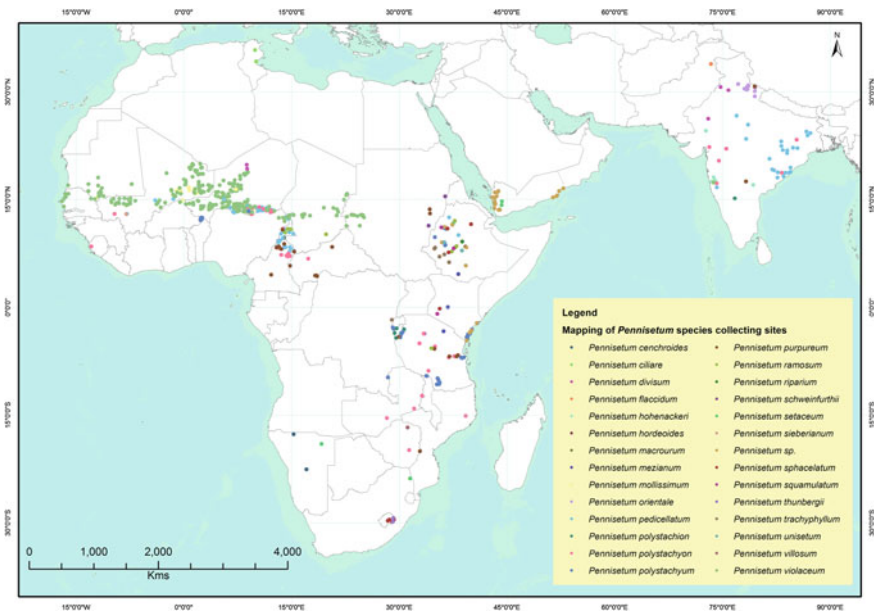


Fig. 3 Mapping of *Pennisetum* species (excluding *Pennisetum glaucum*) accession collecting sites based on information obtained from the global accession level information portal GENESYS, developed by Bioversity International (<http://www.genesys-pgr.org/>)

## 2.2.4 Characterization and Evaluation

From a global survey undertaken by Bioversity International, it appears that although a modest number of accessions have been assembled and maintained in many countries, systematic characterization and evaluation activities are not sufficient. One reason provided by most gene banks is lack of adequate human and financial resources. Evaluation activities, especially in Africa, have been fewer than hoped. In the ICRISAT gene bank, all cultivated accessions have been characterized and evaluated for 23 morphoagronomic characters following the descriptors for pearl millet [75]. Selected pearl millet germplasm accessions of Indian and African origin were evaluated by NBPGR for important agronomic characters at different locations in India in collaboration with ICRISAT and catalogues were published [76, 77]. Considerable phenotypic diversity was observed for almost all quantitative traits. Distribution of qualitative traits indicates occurrence of nine panicle shapes (cylindrical, conical, spindle, club, candle, dumb bell, lanceolate, oblanceolate, and globose), five seed shapes (obovate, oblanceolate, elliptical, hexagonal, globular) and ten seed colors (ivory, cream, yellow, grey, dark grey, grey-brown, brown, purple, purplish-black, and mixture of white and grey) in the entire collection. Accessions with candle-shaped panicles, short bristled panicles, globular seed shape, grey seed color, and seeds with partly corneous endosperm texture are predominant in the collection maintained at ICRISAT [74].

Based on the characterization and evaluation information contained in the database, sources of resistance to biotic and abiotic stresses, adaptation, and nutritional qualitative traits have been identified. It was also reported that some of the landraces have wide adaptation and are therefore very useful in light of the changing climate scenario and for use in crop improvement programs. Much of this diversity is still available in areas of early cultivation in Africa and regions of early introduction in Asia.

## 2.2.5 Utilization

Identification of useful germplasm for crop improvement is the first step in encouraging utilization. From the information obtained through the Bioversity International survey questionnaire, it was difficult to obtain a good comparison of utilization activities being carried out in various genebanks, especially in Africa. However, in India, modest efforts have been undertaken in the last three decades to exploit pearl millet germplasm with useful genes for crop improvement, especially in developing composites. The *iniadi* germplasm from the Togo-Ghana-Burkina Faso-Benin region of Western Africa is most commonly used in pearl millet breeding programs worldwide [78]. At ICRISAT, a small seed sample of each accession is available on request to all research workers under the Standard Material Transfer Agreement of the International Treaty on Plant Genetic Resources for Food and Agriculture [74]. To further enhance the utilization of

pearl millet germplasm, ICRISAT evaluated sets of selected germplasm accessions at different locations in India and several other countries in Africa; trait-specific gene pools (early maturing, high tillering, large panicle, and large grain) were developed to provide partially conserved genotypes to the breeders. There has been a general lack of interest in using wild species because of the large genetic variability in pearl millet landraces. However, some wild species are very useful in pearl millet improvement programs, notably *P. glaucum* subsp. *monodii* for new source of cytoplasmic-nuclear male sterility (CMS); *P. purpureum* for forage, stiff stalk, and restorer genes of the A1 CMS system; *P. orientale* for drought tolerance and forage; *P. schweinfurthii* for large seed; *P. pedicellatum* and *P. polystachion* for downy mildew resistance, and *P. squamulatum* for apomictic gene [48].

In general, the Indian pearl millet landraces have contributed to earliness, high tillering, high harvest index, and local adaptation, whereas African materials have been a good source of high head volume, large seed size, and disease resistance. In order to enhance the use of these genetic resources, Bioversity International, in consultation with various partners, has developed a comprehensive list of descriptors for pearl millet [79]. This strategic set of descriptors, together with passport data, are an integral part of the information available through the global accession-level information portal GENESYS. It will facilitate access to and utilization of pearl millet accessions held in gene banks worldwide.

## 2.2.6 Distribution of *Pennisetum* Species and Gaps in World Collection

A study of the distribution of *Pennisetum* species and of gaps in world collections was undertaken by Bioversity International with support from the Global Crop Diversity Trust (GCDDT) and the World Bank, using datasets of herbarium collections, as well as the germplasm collections available from Global Biodiversity Information Facility and the System-wide Information Network for Genetic Resources and the climate database available at WorldClim. Based on the available records, 53 wild species and 2 infraspecific taxa have been identified, accounting for a total of 55 taxa for the genus *Pennisetum*. These different taxa are classified as follows, according to their closeness to the cropped species *P. glaucum*, using the Maxted and Kell [80] model. The analysis dataset contained 4,326 observations (<http://gisweb.ciat.cgiar.org/GapAnalysis/>) with 3,364 (78 %) being herbarium specimens and 962 (22 %) being gene bank accessions. The average number of total samples per taxon was 79, indicating that available data is not particularly limited, although it is concentrated in certain taxa (i.e. *P. ciliare*, *P. polystachion*, *P. purpureum*, *P. violaceum*, *P. clandestinum*, *P. villosum*). Other taxa such as *P. domingense*, *P. lanatum*, and *P. sieberianum* present a very limited sampling and/or data availability and thus need further characterization and sampling in order to obtain a reliable ecogeographic evaluation.

The gap analysis of the *Pennisetum* gene pool showed that there are 47 out of 55 taxa under analysis that are either underrepresented or not represented in gene banks; these were therefore flagged as high-priority species. Twenty-six of these



taxa presented only 10 data points, which indicates that these species in particular need to be further collected. Only species *P. violaceum* was found to be adequately represented in gene banks, while *P. ciliare*, *P. flaccidum*, *P. orientale*, and *P. pedicellatum* were found to be relatively underrepresented and thus flagged as medium-priority species. Based on the analysis, all these species have been identified as high priority for conservation.

### ***2.3 Quinoa Diversity and Its Potential in the New Millennium***

Quinoa (*Chenopodium quinoa* Willd.) is an ancient native Andean grain that was extensively cultivated in the Andean region by pre-Columbian cultures, such as the Tiahuanacota and Inca, from around 5,000 years ago. This grain was used in the diet of the settlers from the inter-Andean valleys and the high plateaus along with other native species such as potato (*Solanum tuberosum* L.), oca (*Oxalis tuberosa* Molina), amaranth (*Amaranthus caudatus* L.), chili peppers (*Capsicum* sp.), among others [81, 82]. Quinoa is an herbaceous annual Chenopod that has played not only a vital role in family food security and farmer livelihoods, but also in economic, social, ecological, nutritional, and cultural contexts [83, 84]. Quinoa plants are part of various Andean ecosystems; the grains and leaves of this crop are used for food while its subproducts are used for forage, fuel wood, as well as in rituals and handicrafts [85].

This crop, however, began to be marginalized in the sixteenth century with the introduction of cereals such as barley and wheat [81, 86], leading to a significant decrease of quinoa-cultivated areas in the Andean countries [86]. Nevertheless, quinoa cultivation continued in marginal areas. For many decades, quinoa has been considered as a food for the poor and the peasants, but now it is considered to be today's "golden grain". In fact, quinoa was catalogued by FAO in 1996 as "one of the most promissory crops for humanity not only due to its high nutritional value and versatility, but also because it offers alternatives to solve the increasingly serious problems of human nutrition" [86].

Quinoa's key asset lies in its potential as a high-quality source food. Quinoa has high concentrations of some essential amino acids (lysine, methionine, threonine, and tryptophan) that usually limit the quality of human diet. Quinoa is also rich in oligo elements, vitamins, and is gluten free, just to mention some of its nutritional properties [87]. The high content of these amino acids plus the high-quality protein found in its grain can cover the nutritional requirements for schoolchildren and even adults [88], which are serious problems in some populations around the world.

The Andean region is one of eight centers of origin and diversity of cultivated plants in the world described by Vavilov in 1953; that is, it is one of the regions that has the highest diversity of cultivated crops and their wild relatives. In this region and specifically around Lake Titicaca, a high plateau region in Bolivia and Peru, the highest genetic diversity of wild and cultivated quinoa genotypes are found in farmer fields [81, 89, 90].

Agriculture in the Andean highlands is characterized by a high degree of risk due to a range of harsh climatic factors such as frost, hail, wind, drought, high radiation, and poor and saline soils [91]. It is highly probable that during domestication, Andean farmers selected certain genotypes based on their use and their tolerance to adverse biotic and abiotic factors, thereby obtaining current plants and ecotypes that possess high diversity of traits. Some of these ecotypes have been strictly selected as a source of food (finding up to hundred different recipes), while others have been selected based upon their tolerance to salinity, poor soils, cold climate, frost and hail, drought, and flooding. It is important to remark that some genotypes have also been selected due to their high yield potential and precocity [89, 92, 93].

During the domestication of quinoa by Andean farmers, a wide range of morphological modifications have occurred to the plant, such as the condensation of the inflorescence in the highest part of the plant, an increase in plant and seed size, reduction of the testa, loss of seed dormancy and dispersion mechanisms, and high variation in levels of pigmentation, among others. Today, we can find quinoa plants with high production of larger seeds and clear colors; these traits reflect the long time man has been using, selecting, and cultivating this species [93]. Although this species has been completely domesticated, the seeds still contain saponin, which must be extracted before consumption [86]. Quinoa ancestors and relatives still exhibit the wild traits of the crop [93].

The genetic variability of quinoa is huge [91]. For example, one of the most popular varieties grown today in Bolivia (*Quinoa real*) has at least 73 ecotypes. This variety is extremely well adapted to saline environments and has big grains. In addition, 47 local landraces and approximately 10 improved varieties are also found (Table 1).

In other quinoa-producing countries within the Andean region, a considerable diversity of local and improved varieties can also be found [86, 92]. The existence of this vast amount of ecotypes, improved and local varieties, and/or landraces are the result of the intense breeding processes, domestication, and use and conservation practices adopted by ancient and contemporary Andean farmer communities based on their traditional knowledge [86].

Currently, the demand for quinoa in the national and international markets has increased; quinoa production areas have also shown this tendency due to high market prices. However, this demand has preference for larger seed size, uniform color and white color, and saponin-free seeds [93, 94]. This has progressively caused the domination of a group of cultivated varieties with these traits, such as various ecotypes of *Quinoa real* [89, 94]. Although markets have preferred these varieties, Bonifacio et al. [94] reported that, in the last couple of years, those with red and brown seeds have also been accepted now in some markets, although not to the same extent. Nevertheless, this market preference can be risky as it can further lead to genetic erosion and the loss of some varieties. Unfortunately, this process is in progress, and it has been stated that three varieties of quinoa and a

**Table 1** Common names of varieties, landraces, or ecotypes found in Bolivia

| Varieties or landraces | Common name   |
|------------------------|---|
| Quinoa Real            | Achachino <sup>a</sup> , achachino rojo, café chullpa, canchis/qanchis blanca, canchis roja, carequimeña, ch'illpi amapola, ch'ullpi blanco, ch'ullpi rojo, ch'ullpi rosado, chachahua, challamoko, challamuro, chhuku puñete, chillpi, chipaya, hilo, huallata/wallata, imilla, intinayra <sup>a</sup> , jiskitu, kairoja, kellu/q'illu <sup>a</sup> , lipeña, mañiqueña, mañiqueña nor lípez, mañiqueña palaya, manzano, mok'o rosado/moqu rosado, moqu, moqu chacala, mururata, negra, negra blanquita, negra blanquita planta roja, pandela/rosada <sup>a</sup> , pandela amarilla, perlasa, phisanqalla 3 hermanos, phisanqalla amarantiforme, phisanqalla hembra, phisanqalla macho, pisankalla (ayrampu)/pasankalla <sup>a</sup> , pucayua, puñete, punta blanca, q'illu puñete, q'uitu/koitu, q'uitu rojo, qanchis amarillo, qanchis anaranjado, qanchis rosado, qhaslala blanca, quinoa roja, real blanca/real <sup>a</sup> , romerilla, rosa blanca, santa maría, señora, sorata, tacagua, timsa/timza 1, timza nor lípez, toledo <sup>a</sup> , toledo anaranjado, toledo rojo, tres hermanos/siete hermanos, tupita, ucaya, utusaya, utusaya local chacala, wila jipiña, wilalaca |
| Other local landraces  | Acu jaira, ajara (wild), amarilla, amarilla maranganí, arroz jupha, blanca, blanca de july, choq'e pito, chuchi jiura, churo iri, cochasqui, coytu, cuntur naira, elva, granadilla, imbaya, ingapirca, iry, janko cayun jaira, janko jhupa, juchuy mojo, kaslala/kaslali matizada, katamari, kcancolla, kelly jaira, llulluchi, mezcla, misa jupa, mixtura, negra, negra de oruro, noventona, palco, ploma, pureja, quilliwillu, roja, roja coporaque, sallami, siki, tunkahuan, waca misu, waranta, wila cayun janq'o, wilacoimi, witulla, yubi  |
| Improved varieties     | Chuca paca <sup>a</sup> , jacha grano, jilata, kamiri, ratuqui, robura, sajama <sup>a</sup> , samaranti, sayaña <sup>a</sup> , surumi <sup>a</sup>  |

<sup>a</sup> Most commonly used by communities

Sources [89, 93, 94, 195–197]

wild relative have already been included in the International Union for Conservation of Nature red list categories as endangered (EN), near threatened (NT) and least concern (LC) [95].

Apaza et al. [96] reported that the highest diversity of quinoa is found in *aynokas*, *mandas*, and *laymes*, a group of traditional organization systems or communal fields maintained by farmer communities in field borders and sacred places (all called *Gentil wasi* or *Phiru*) using local cultivation methods and traditional knowledge. If these places continue being eroded and the traditional practices and knowledge become lost by the adoption of modern culture practices, it is highly probable that the diversity present in these sites will disappear.

To conserve the enormous genetic diversity of quinoa and some of its wild relatives, various germplasm banks were created in the 1960s throughout the South American region. These banks are being managed by public or private entities interested in the *ex situ* conservation of plant genetic resources, thereby finding more facilities in countries with higher diversity (Table 2). Collections of quinoa germplasm began in this period with the help and sponsorship of various private and public organizations, donors, and projects that still subsist today.

**Table 2** Approximate number of quinoa accessions conserved in *ex situ* facilities across South America

| Country   | Number of entities that conserve quinoa germplasm | Number of accessions conserved (countries of origin)   | Access to the material |
|-----------|---|--|------------------------|
| Argentina | 2   | 63 (Argentina, Bolivia, Chile, Ecuador, Peru, U.S.A.)  | Yes                    |
| Bolivia   | 8   | More than 7,077 (Bolivia, Peru, Ecuador, Colombia, Argentina, Chile, Mexico, U.S.A., Denmark, Holland and England) | Yes                    |
| Chile     | 3   | 152 (Chile)  | Yes                    |
| Colombia  | 3   | More than 328 (Colombia, other)  | Yes                    |
| Ecuador   | 1   | 608 (Ecuador, Bolivia, Peru, Colombia, Argentina)  | Yes                    |
| Peru      | 12  | More than 4,431 (Peru, Bolivia, Colombia, Ecuador)   | Yes                    |

Sources [86, 198, 199]

Although these banks keep an important number of quinoa accessions, the variability stored in these collections do not represent all the Andean diversity [86], especially when it comes to wild populations and wild relatives [97]. There are at least ten wild species or wild relatives and a couple of subspecies of the *Chenopodium* genus, known as wild quinoa or *ajara*, that are used for certain recipes by Andean farmers. These wild species are not being properly conserved.

Since 2001, some quinoa varieties and wild relatives, such as *cañahua* (*Chenopodium pallidicaule* Aellen), have been evaluated for yield, harvest index, postharvest, market, industrialization potential, new products and uses, among other fields, within the framework of the International Fund for Agricultural Development Neglected and Underutilized Species–Biodiversity International Project 2001–2014. Additionally, other wild relatives are being evaluated for their potential to improve the tolerance to biotic and abiotic stresses as part of a United Nations Environment Programme/Global Environment Facility project for nutritive properties [90].

To date, results show that there is a huge potential in the conservation and use of quinoa diversity and its wild relatives. Studies also show that there is considerable variation among cultivars for a wide range of traits. This fact allows users to take advantage of quinoa's versatility for use in more than 100 different preparations and products. Other than its cosmetic and industrial and pharmaceutical uses, quinoa can also be used as forage, medicine, pesticide, ornament, and fuel wood [91, 93, 98]. In addition, quinoa has a promising market potential for high-value grain, subproducts, and byproducts that still remain unexploited [99].

Both quinoa and its wild relatives possess an enormous reservoir of genetic variation that can be exploited through plant breeding. They are also an essential resource to meet the challenge to improve food security, enhance agricultural production, and sustain productivity in the context of a growing world population

and the threats of climate change [90, 100]. Likewise, quinoa diversity can be greatly exploited through biotechnological approaches to the benefit of agricultural industries [98].

#### ***2.4 A Practical Use of Agrobiodiversity in Cereal Crops to Mitigate Climate Change through Regulation of Soil Nitrification***

It has been shown repeatedly how the exploration of the natural diversity in cereals -and in crops in general- has been the solution for multiple challenges that naturally arise as a result of agricultural practices (tolerance to biotic and abiotic stresses, yield increments, adaptation to different conditions other than the ones in the center of origin, etc.). Along with the need of yield increments in the main staples cereals -as the global population is exponentially growing-, climate change is a prominent issue that is challenging agriculture and even mankind. Agriculture is an important source of anthropogenic emissions of the greenhouse gases (GHG) methane ( $\text{CH}_4$ ) and nitrous oxide ( $\text{N}_2\text{O}$ ) associated with nitrogen (N) fertilizer production [101]. It is now known that  $\text{N}_2\text{O}$  has a higher ozone-depletion potential than any other reactive chemical, including carbon dioxide [102], and also that agricultural practices are the major source of  $\text{N}_2\text{O}$  emissions to the atmosphere [103]. Under this scenario, a disjunctive stands, either to continue practicing agriculture for food and feed in the way we are currently doing or to look into alternatives to implement a “climate-smart” agriculture, where we as humans not only aim to feed the world but also care for the environment. Once again, researchers have found that natural diversity in certain crops confer characteristics to some lines that are “eco-friendly” and that, for example, reduce the emission of GHG as  $\text{CH}_4$  and  $\text{N}_2\text{O}$  [104].

Much of the  $\text{N}_2\text{O}$  produced by agriculture is generated from the use of N fertilizers that, after application to soil, feed the nitrification reaction; in this process, nitrifying microorganisms take ammonium and convert it into nitrite and finally into nitrate, a compound susceptible to leaching, thereby contaminating bodies of water. Nitrate is eventually reduced by denitrification, releasing  $\text{N}_2\text{O}$  gas as a byproduct [105]. Researchers from the International Center for Tropical Agriculture and the Japan International Research Center for Agricultural Sciences observed that the tropical pasture *Brachiaria* spp. has the ability to inhibit the soil nitrification process by releasing chemical compounds from its root system to the soil; a compound with major inhibition capacity was identified and named brachialactone [106]. This phenomenon was termed biological nitrification inhibition (BNI) [107]. The BNI trait would decrease costs for the farmer because the N applied will stay longer in the soil and the plants would have a better chance to intake it before it is converted into forms that are prone to leaching and losses via gaseous forms, thereby improving the ecoefficiency of agricultural practices by

diminishing the amount of  $N_2O$  emissions that are released to the atmosphere. Subbarao et al. [106] demonstrated under field conditions that plots of *Brachiaria* pastures significantly reduced  $N_2O$  emissions when compared to bare soil, soybean, and guinea grass plots.

After the BNI concept was brought to light, researchers identified the great potential that this phenomenon has in agricultural practices by reducing the N contaminants (i.e., nitrate and  $N_2O$ ); therefore, this strategy can be applied to mitigate global warming largely occasioned by GHG. For that reason, Subbarao et al. [108] achieved and attempt to identify in which other plant species the BNI potential was presented, as well as to what extent there was genetic variability of the BNI potential in cereal crops. The outcome of this investigation showed that BNI is widely existent in pasture grasses, but the investigation also reported that the roots of two cereal crops *Sorghum bicolor* (L.) Moench var. hybrid sorgo and *Pennisetum glaucum* (L.) R. Br. var. CIVT (Pearl millet) release chemicals to the soil that reduce the population of nitrifier microorganism and therefore inhibit the nitrification rates. Other cereals, such as rice, maize, barley, and wheat, were also tested in this preliminary screening with negative results (no BNI activity detected), but in most cases using single lines; therefore, it is recommended to test a diversity panel for each species to really inspect the BNI trait on each cereal crop. Tanaka et al. [109] tested the BNI activity in the root exudates of 36 different rice genotypes and found substantial genotypic variation for BNI, with the upland cultivar IAC25 expressing high BNI activity, whereas lowland varieties such as Nipponbare or IR64 exhibited low BNI activity.

Although initial evaluations in wheat showed that the roots of this cereal do not exudate chemical compounds to the soil that successfully control the nitrification process, further analysis of *Leymus racemosus* (mammoth wild rye, a wild wheat relative), resulted in the discovery of a high BNI capacity that efficiently suppressed soil nitrification [110]. The exploration of genetic diversity of wild predecessors of wheat showed how the BNI trait has been lost in the course of decades of breeding and selection of “desired” (at a given time and condition) agronomic conditions that inevitable lead to accidental loss of other valuable traits. As a result, the chromosomal location of the genes conferring the BNI trait has been identified and wheat varieties expressing the BNI trait have been formed by the production of wheat–*Leymus racemosus* chromosome addition lines [111].

The cereal crop in which significant BNI research has been conducted is sorghum, and both hydrophilic and hydrophobic root exudates with BNI activity have been identified [112, 113]. An ample natural diversity has also been identified among sorghum lines for the release of sorgoleone (a major hydrophobic root exudate with the highest BNI capacity). Nimbal et al. [114] evaluated sorgoleone production among 25 sorghum lines, finding nearly a 30-fold variation. Current efforts will focus on the exploration of sorghum diversity to exploit the BNI trait and to identify sorghum genetic stocks with high potential to release chemicals to the soil that suppress nitrification, reduce  $N_2O$  emissions, and improve nitrogen use efficiency in sorghum-based production systems [113].

Cereal production uses the most of N-fertilizers; wheat itself accounts for a third of the global production [115] to cope with the food demand of a growing population. The indiscriminate use of N fertilizers results in N contaminants (e.g. nitrate and nitrite) that are causing major problems to bodies of water and are also contributing to global warming. BNI function represents a novel opportunity to naturally establish agricultural systems with improved N use efficiency and reduced N-contaminants. Therefore, the exploration of diversity of major cereal crops is imperative to promote the ecoefficiency of agricultural systems.

## ***2.5 Impact of Nitrogen Pollutants on Biodiversity***

Biodiversity is declining at an exceptional rate and on a worldwide scale. Indeed, loss of ecosystem functions and services associated with such declines has generated international debate [116–118]. Agricultural crops can be injured when exposed to high concentrations of various air, water, and soil pollutants. Air pollution affects plants in many ways, which have implications for overall biodiversity and ecology. There is evidence that air pollution can reduce some plants' ability to reproduce, thus causing long-term changes to population ecology [119]. Of the different kinds of pollutants damaging the environment, nitrate pollution is a major problem along with the pollution of the atmosphere by ammonia and oxides of nitrogen. Nitrogen is a beneficial plant fertilizer in small amounts, but large amounts cause negative impacts on ecosystems and serious threat to biodiversity of many groups of organisms, including diversity of plants [120–122]. Nitrogen deposition refers to the input of reactive nitrogen species from the atmosphere to the biosphere. At the global scale, current N emission scenarios project that most regions will have increased rates of atmospheric N deposition in 2030 [123], which is causing concern about significant impacts on global plant biodiversity [116, 117, 124].

Even though low-to-medium levels of N addition ( $\leq 100$  kg N/ha/year) generally did not alter plant diversity through time, high levels of N application significantly reduced species diversity [125]. The declines of diversity appeared to arise from N-related changes in soil properties, such as significant decreases in pH and extractable calcium (Ca) and increases in extractable aluminum (Al). Research reports revealed that N deposition may have shifted plant communities towards species composition typical of high-N availability. This shift has often been associated with a loss in diversity of plant species, particularly in areas with high deposition rates [126]. Mechanisms underlying the declines of diversity include competitive exclusion of more N-efficient dominant species by relatively fast-growing nitrophilic species, as a result of high-N availability induced by N deposition. Other such mechanisms include increased susceptibility to secondary stress and disturbance factors and species invasions [127, 128]. The research review of N-addition experiments across the tropics and subtropics have shown that N deposition may potentially affect plant diversity in some ecosystems more

than originally thought, and because atmospheric N loads are gradually increasing in some tropical areas such as Asia, research on this topic is now urgently required [127].

Although improved agronomic approaches are one way to enhance nitrogen use efficiency (NUE) and reduce the N inputs, there is a growing interest in understanding the genetics of NUE in crop plants. Integration of these approaches may reduce both N deposition and the rapid loss of biodiversity.

## 2.6 *Transgenic Crops and Food Security*

The development of technologies for plant transformation started in the early 1980s following the production of chimeric genes [129–132], transformation vectors [133, 134], and DNA delivery systems [135–139], combined with plant tissue culture regeneration systems, which were pioneered by Murashige and Skoog [140]. This continued over the decades with the development of tissue culture for the three most important cereals: rice [141, 142], maize [143, 144], and wheat [145, 146]. The transformation of cereals since the 1990s was achieved with *Agrobacterium*-based protocols for rice [147], maize [148], and wheat [149], usually with a low-copy insertion of transgenes [150–152] and the *gus* reporter gene [153] with antibiotic selectable marker genes [154, 155]. These techniques were the most widely used in earlier transgenic plant research and crop development. Currently, more than 50 selectable marker genes are available for transgenic research and commercialization, and they can be divided into several categories depending on the mode of action and substrates used [156–158].

An important milestone in plant transgenic technology is the ability to generate plants free of the selectable marker gene using co-transformation with multiple T-DNAs [159, 160]. Among the major advances in plant transformation technology, we can consider the understanding of the plant cell–*Agrobacterium* relationship [161] and the molecular mechanisms of T-DNA transfer [162, 163] together with integration into the plant genome [164, 165].

Vain [166, 167] reviewed publication trends in and scientific knowledge on plant transgenic science and technologies available worldwide. In addition, the security of transgenic plants and products has been scrutinized [168–172], including the economic, environmental, and social dimensions [173].

The newest discoveries describing gene silencing [174], gene targeting [175], and RNA interference [176, 177], with alteration of the transcriptional activity of genes by using zinc-fingers nucleases [178, 179], together with the possibility of genome editing by TALENs (transcription activator-like effector nucleases) [180, 181], including the technology of using site-directed nucleases, are providing an opportunity to develop a new generation of transgenic crops with both improved traits and minimized potential for unintended effects that can impact safety [182].

It is very well known that agricultural production in the twenty-first century is going to face a number of new challenges [183–186]. A staple crop such as rice,



for which more than half of the global population already depends on its production, will be under more and more pressure to increase its yield steadily; this has happened during the last four decades in some countries, where rice yield doubled or tripled [187]. New and multi-integrated strategies, including functional genomics, phenomics, and transgenics, coupled with conventional rice breeding, can help to develop new rice cultivars (referred to by Zhang [188] as Green Super Rice) and can meet future demand for world rice production. Take into consideration the fact that, from 1996 to 2012, millions of farmers in 30 countries worldwide adopted biotech/genetically modified organism (GMO) crops at an unprecedented level, going from 1.7 million ha in 1996 to 170 million ha in 2012 [189], a 100-fold increase. In addition, Golden Rice [190] is expected to be released in the Philippines in 2014, together with drought-tolerant sugarcane in Indonesia, biotech maize [191], and rice [192] in China. As a result, up to a billion poor people in rice households in Asia could benefit. Beyond 2015, it is difficult to predict what will come to the market, but expectations are also high in Africa, where the private/public partnership Water Efficient Maize for Africa [193] will release the first biotech drought-tolerant maize in the sub-Saharan region, where the need for drought-tolerant crops is greatest.

Commercialized crops could help to reduce the impacts of agriculture on biodiversity by alleviating pressure to convert additional land into agricultural use [194].

### 3 Summary and Conclusions

The management of plant genetic resources for food and agriculture is becoming a central issue in the context of sustainable agriculture, climate change, and food security. As the world faces unprecedented challenges, the exploration, use, and conservation of agricultural biodiversity will play a major role to address the most critical socioeconomical and environmental issues concerning agricultural research and food security strategies. The agricultural biodiversity of staple small grains, such as rice, maize, wheat, millets, and quinoa, is currently being studied from several perspectives to offer information to researchers and breeders worldwide.

Only a few accessions of pearl millets have been maintained so far in several countries. In-depth characterization and evaluation of these accessions remain insufficient due to lack of human and financial resources. Nevertheless, significant phenotypic diversity has been observed for most quantitative traits in a set of selected pearl millet germplasm accessions. This diversity is therefore very useful for use in crop improvement programs. Although the genetic variability in pearl millet landraces is large, some wild species have also been considered as a source of genes controlling agronomically important traits.

In the light of land change uses, modernization of agricultural practices, and climate change, which eventually affect plant diversity patterns, transnational efforts have focus on the establishment of extensive genebank collections for cereal crops. Both *in situ* and *ex situ* conservation of plant genetic resources are

complementary strategies for the preservation of agricultural biodiversity. Geospatial analysis tools can be applied to map microsatellite diversity of cereal accessions characterized molecularly in order to identify priority geographic areas for conservation.

Although quinoa and its wild relatives are a reservoir of rich genetic variation that can be used by breeders for further improvement of the species, quinoa diversity is being lost. Market demand has encouraged the cultivation of commercially valuable varieties with certain characteristics, neglecting the immense variation found within the species. Some local quinoa genebanks have been created, containing many known quinoa varieties with a wide range of diverse agronomic and nutritional properties. However, current quinoa genebanks still do not represent the Andean diversity of the cultivated and wild relatives.

Agrobiodiversity can provide additional sources of new traits. Some cereal crops have the ability to inhibit soil nitrification and reduce the emissions of greenhouse gases (BNI activity) by releasing root exudates. The screening of some cereal accessions revealed genotypic variation for BNI. The exploration of agrobiodiversity for the BNI trait offers a novel strategy to improve the nitrogen use efficiency and increase crop productivity while reducing the environmental impacts of agriculture.

Chemical pollutants that are released into the environment are posing a threat to plant biodiversity. Nitrogen pollution is a major problem worldwide due to the indiscriminate use of synthetic nitrogen fertilizers in agricultural systems. This practice affects the natural balance of soil ecosystems, leading to significant losses of plant biodiversity as a result of changes in soil properties and plant community structure. Research addressing the genetics of nitrogen use efficiency in crop plants plus the adoption of sustainable agronomic practices offer an integrated approach to minimize the negative impact of reactive nitrogen on plant biodiversity and the environment.

Integration of transgenics, genomics, proteomics, and conventional plant breeding strategies can greatly accelerate the development of new cereal cultivars. Other than increasing crop productivity, commercialized genetically modified crops could help to reduce the impacts of agriculture on biodiversity by alleviating pressure to convert additional land into agricultural use.

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