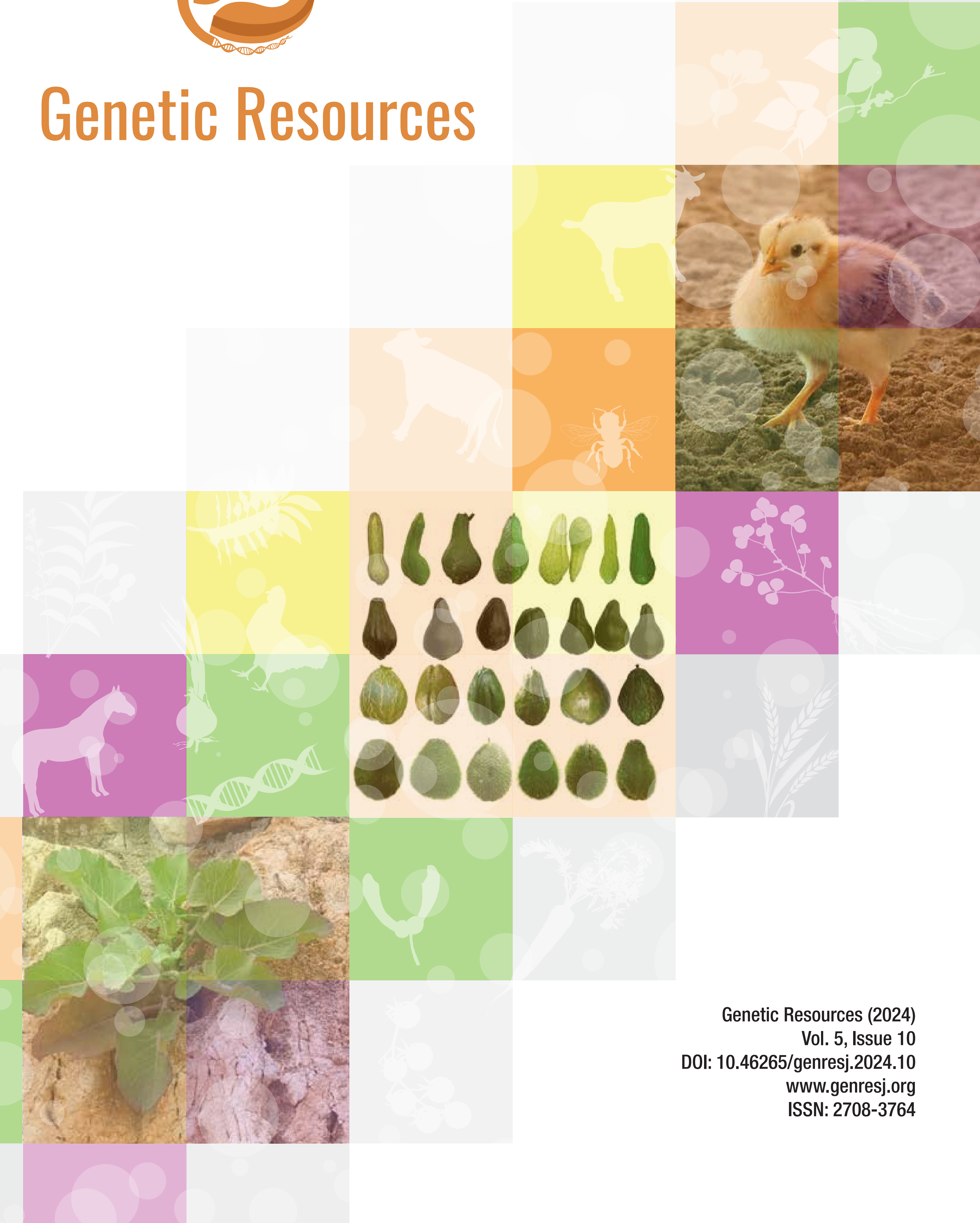
Genetic Resources



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Focus and Scope of *Genetic Resources*

Genetic Resources is an open access journal disseminating global knowledge and tools used by the community of practitioners of plant and animal genetic resources involved in monitoring, collecting, maintaining, conserving, characterizing and using genetic resources for food, agriculture and forestry. ***Genetic Resources*** publishes original research, methods, strategies, guidelines, case studies and reviews as well as opinion and other papers on a variety of topics of interest on the present and future use of genetic resources. These may include the acquisition, documentation, conservation, management, assessment, characterization and evaluation of genetic resources and their link to broader biodiversity, socioeconomic practices, policy guidelines or similar, serving stakeholders within and across sectors. Occasionally, ***Genetic Resources*** publishes special issues with a focus on selected topics of interest for the genetic resources community. The journal has a focus on the European region and also welcomes contributions of wider interest from all world regions.

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Brassica cretica Lam, credit: Sokrat Jani; Chayote diversity, Mexico, credit: J. Cadena-Iñiguez *et al*; Chicks, Ghana, credit: Bioversity International/C. Zanzanaini.

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ORIGINAL ARTICLE

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Collecting Mediterranean wild species of the *Brassica oleracea* group (*Brassica* sect. *Brassica*)

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Abstract: Within the framework of the project EUBRASWILD (Capturing *Brassica* Wild Relatives Diversity in Southeastern Europe), several collecting missions were organized, targeting wild *Brassica* complex species (2n = 18), belonging to the gene pool of *Brassica oleracea* L. These crop wild relatives have repeatedly shown their potential to contain useful alleles for biotic and abiotic stress resistance, and nutritional or health-beneficial traits that can be easily intercrossed with the related crop. The missions described in this paper aimed to collect taxa that are poorly represented in public genebanks or databases for long-term conservation. This report describes missions carried out by national teams in Albania, Croatia and Italy (Ponza and Sicily), including highlights of newly discovered locations.

Keywords: *Brassica cretica, Brassica drepanensis, Brassica incana, Brassica macrocarpa, Brassica montana, Brassica oleracea, Brassica rupestris, Brassica villosa*, Crop wild relatives, Collecting missions

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Introduction

The cole crops (*Brassica oleracea* L.) comprise several types of commercially important vegetables, including cabbage, cauliflower and broccoli (Branca, 2008). These crops share the same C genome with 2n = 18 chromosomes and easily intercross with several wild species native to the Mediterranean area, such as *B. cretica* Lam., *B. drepanensis* (Caruel) Damanti, *B. incana* Ten., *B. insularis* Moris, *B. macrocarpa* Guss., *B. montana* Pourr., *B. rupestris* Raf., *B. villosa* Biv., included in *Brassica* section *Brassica* (Snogerup *et al*, 1990; Bothmer *et al*, 1995). These crop wild relatives are potential providers of agronomically useful traits and can serve as a source of suitable alleles that may have been lost during the domestication process. For example, in *B. incana*, resistance against various fungal diseases has been identified, such as Verticillium wilt (*Verticillium longisporum*) and Sclerotinia sclerotiorum (Happstadius *et al*, 2003; Mei *et al*, 2011; Taylor *et al*, 2018); in *B. insularis* against *Leptosphaeria maculans* (Mithen and Magrath, 1992) and *Pyrenopeziza brassicae* (light leaf spot disease) (Bradburne *et al*, 1999); in *B. villosa* against downy mildew (*Hyaloperonospora brassicae*) (Coelho

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et al, 2018). Recently, resistance to *Xanthomonas campestris* was identified in one accession of *B. montana* collected in 2005 by L. Maggioni on the island of Ponza, Italy, differently from what was reported in Sheng *et al* (2020) (F. Branca, pers. comm.). Various types of insect resistance were studied in *B. villosa*, *B. incana* and *B. montana* (Palaniswamy and Bodnaryk, 1994; Ellis *et al*, 1999; Pelgrom *et al*, 2015; Vosman *et al*, 2015). Furthermore, Zhang *et al* (2016) carried out a comparative transcriptome analysis to evaluate the resistance against *Plasmodiophora brassicae* in broccoli and *B. macrocarpa*. Their analysis revealed the activation of different metabolic pathways in *B. macrocarpa*, which enabled it to overcome the pathogen infection. The opportunity to breed for higher content of potentially beneficial (antioxidant and anti-cancer) glucosinolates has been studied (Picchi *et al*, 2020; Arena *et al*, 2022; Bianchi *et al*, 2024) and used for the development of the 'Beneforte' broccoli, resulting from a cross with *B. villosa* from Sicily (Mithen, 2014). The wide genetic variability expressed by *Brassica* wild relatives has also facilitated the detection of distinct allelic patterns associated with morphometric traits in wild relatives compared to their cultivated counterparts (Treccarichi *et al*, 2023).

The importance of wild relatives of *B. oleracea* and the high priority for their seed conservation were highlighted as far back as 1981 in a plan of action report prepared by international experts for the International Board for Plant Genetic Resources (IBPGR), with the title *Genetic Resources of Cruciferous Crops* (IBPGR, 1980). A plan of action was subsequently accepted and supported by IBPGR as the 'Germplasm Conservation of Wild (n = 9) Mediterranean Brassicas Project' (Gustafsson, 1982; Gustafsson *et al*, 1985). Six collecting missions were funded between 1982 and 1988 targeting Cyprus, France, Greece, Italy, Spain, Tunisia, Turkey and the United Kingdom (Gómez-Campo *et al*, 1994). An agreement was signed in 1981 between IBPGR and the genebank of the Universidad Politécnica, Madrid (UPM), to hold a global collection of wild relatives of cruciferous crops, as part of the register of base collections, with agreed responsibilities for long-term conservation and distribution to bona fide users (Maggioni, 2010). Therefore, all samples were deposited at UPM, as well as safety-duplicated in the country of origin and other genebanks.

Despite these arrangements, a survey of the status of *Brassica* wild relative accessions (Maggioni *et al*, 2013) revealed that most species and localities were underrepresented in collections. The main geographical gaps were the Adriatic coasts (Albania, Croatia and Italy), the Aegean coasts (Greece and Turkey), northern Cyprus and the coastal cliffs of Tunisia. Moreover, the availability of accessions with a Standard Material Transfer Agreement (SMTA) was very limited, owing to the lack of sufficient seed, since brassicas are laborious and expensive to multiply. In other cases, there was reluctance to share wild brassicas, even though the entire *Brassica* complex is fully included in Annex I of the International Treaty on Plant Genetic Resources for Food and Agriculture (FAO, 2009).

In particular, UPM became unable to guarantee sufficient multiplication and distribution of samples (Gonzalez-Benito, 2010). Similar difficulties were faced by other genebanks. Additionally, some geographic areas had not been covered by the international collecting missions, such as the Croatian coast, observations of new sites have constantly expanded the known distribution range of these wild relatives, and new species were proposed, such as *B. tyrrhena* Giotta, Piccitto & Arrigoni (Giotta *et al*, 2002) and *B. trichocarpa* C. Brullo, Brullo, Giusso & Ilardi (Brullo *et al*, 2013). The most comprehensive distribution map and list of observations of all the C genome wild *Brassica* species still remains the one prepared by Snogerup *et al* (1990), although it is today outdated and would require a thorough revision.

A small project titled 'Capturing *Brassica* Wild Relatives Diversity in Southeastern Europe (EUBRASWILD)', funded by the European Cooperative Programme for Plant Genetic Resources (ECPGR), was recently set up under the coordination of Smiljana Goreta Ban, Institute of Agriculture and Tourism, Poreč, Croatia, involving partners from seven countries of the south Balkan area and Italy. Among the objectives of this project, which was dedicated to monitor, collect and characterize various populations of crop wild relatives in the Brassicaceae family, there was also the exploration, collecting and multiplication of (2n = 18) wild *Brassica* populations. This paper describes the collecting missions carried out in Albania, Croatia and Italy, including background information, methodology and results, focusing on *B. cretica*, *B. drepanensis*, *B. incana*, *B. macrocarpa*, *B. montana*, *B. rupestris* and *B. villosa*. Knowledge about the distribution range and existing collections in Europe and their gaps for the above species is briefly summarized below. Taxonomy names and synonyms are according to the GRIN Taxonomy (Wiersema and Schori, 2022) and Euro+med (2006) for taxa not treated by GRIN.

Brassica cretica Lam.

[Synonym: *Brassica oleracea* L. subsp. *cretica* (Lam.) Gladis & K.Hammer]

The distribution range of this species covers mainly coastal areas of Albania, Greece, west Turkey, central and south Lebanon (possibly introduced) and Israel at Mount Carmel (also possibly introduced) (Snogerup *et al*, 1990; Barina *et al*, 2011; Flora Ionica Working Group, 2016). Collections documented in the European Search Catalogue for Plant Genetic Resources (EURISCO, http://eurisco.ecpgr.org), consist of 120 accessions, conserved in Greece (43), Spain (42), Albania (17), the United Kingdom (10), Germany (5), Israel (2) and Italy (1). These originate from Greece (85), Albania (17), Turkey (10), Israel (3) Lebanon (3) and unknown (2). Accessions from Albania have recently been added to the Albanian Genebank as a result of the EUBRASWILD activity, whereby a thorough exploration has filled gaps in knowledge and material, as described in this paper. Other areas of Greece and Turkey are not well covered by the documented collections of this species.

Brassica drepanensis (Caruel) Damanti

[Synonym: *Brassica villosa* Biv. subsp. *drepanensis* (Caruel) Raimondo & Mazzola]

This taxon is endemic to northwest Sicily, Italy, and limited to four locations in the province of Trapani, with an 'endangered' status, according to the IUCN Red List of Threatened Species (Maggioni and Domina, 2020). Urbanization and fires are likely to threaten all known subpopulations of this species, especially below the cliffs of Mount Erice (Snogerup *et al*, 1990). Only thirteen accessions clearly referable to this taxon are documented in EURISCO. Four of these are conserved at IPK in Germany, four at UPM Madrid, Spain and one in Warwick, United Kingdom, all collected around the town of Erice. One sample is conserved at the University of Catania, Italy, received as a duplicate from IPK, Germany. Other three accessions, with undisclosed location of collecting, are conserved in Hungary (2) and the United Kingdom (1). Two accessions documented under the name *B. villosa* subsp. *drepanensis*, conserved at UPM, Spain, were collected outside the recognized distribution area of this taxon, which raises doubts about their correct identification. The population in Erice has been the most collected, which is justified by the fact that it is relatively small and probably the most threatened due to human activities. Other small populations are included in protected areas (Zingaro Nature Reserve and Monte Cofano, Capo San Vito e Monte Sparagio Special Protection Area) (Raimondo *et al*, 1991). However, they are not subject to active conservation and are not regularly monitored. Therefore, they would deserve to also be secured in a genebank. Although local collections of these populations may exist in Sicilian universities or botanic gardens, the Italian national inventory displayed in EURISCO is deficient as far as *B. drepanensis* is concerned.

Brassica incana Ten.

[Synonyms: *Brassica botteri* Vis.; *Brassica cazzae* Ginzb. & Teyber; *Brassica mollis* Vis., *Brassica taurica* (Tzvelev) Tzvelev]

This species is distributed in Tyrrhenian coastal areas of central and south Italy, northeast and east Sicily, Adriatic coastal localities in Puglia and Croatian islands, south Albanian coast and Greek Ionian islands (Snogerup *et al*, 1990; Baldini, 1995; Castellano and Bazan, 2009; Anzalone *et al*, 2010; Barina *et al*, 2011; Maggioni, 2015; Flora Ionica Working Group, 2016; Bartolucci *et al*, 2018). One population growing in Crimea, farther apart from the rest of the distribution range, has been considered as an introduced sample (Snogerup *et al*, 1990) and was interpreted as a feral lineage by Mabry *et al* (2021). Based on EURISCO, 53 accessions are conserved in European genebanks in Spain (28), the United Kingdom (11), Germany (7), Albania (5) and Italy (2). Overall, these samples originate from Italy (37, of which 17 from Campania, 16 from Sicily, 2 from Tuscany, 1 from Lazio and 1 from Puglia), Albania (5), Ukraine (3), Croatia (1) and unknown (7). This collection appears largely unrepresentative of the diversity and geographic distribution of this species, with several gaps from the Italian distribution area, as well as from Croatia and Greece, the latter two totally missing, except for one sample from Korčula, Croatia. Particularly poor is the collection maintained in Italy, which declares to conserve only two samples, even though the country hosts the widest number of existing populations within its territory.

Brassica macrocarpa Guss.

[Synonyms: *Brassica oleracea* L. subsp. *macrocarpa* (Guss.) Gladis & K. Hammer; *Eruca macrocarpa* (Guss.) Caruel]

This is an endemism of Egadi Islands, Sicily, which is present only on the two islands of Favignana and Marettimo, while it has no longer been found on the island of Levanzo (Maggioni *et al*, 1996). This species is classified as critically endangered in the IUCN Red List of Threatened Species, due to its very narrow extent of occurrence, area of occupancy and risk of decline due to the possible human disturbance of its habitat (Branca and Tribulato, 2011). According to EURISCO, 23 accessions are conserved in Germany (2), Spain (4) and the United Kingdom (17). Most of them were collected in Favignana and two of them are from Marettimo.

Brassica montana Pourr.

[Synonyms: *Brassica robertiana* J. Gay; *B. oleracea* L. subsp. *robertiana* (Gay) Rouy et Foucaud]

B. montana is distributed along the northern Mediterranean coasts of Spain (Gerona province), south France and Italy (Liguria and northern Tuscany) (Snogerup *et al*, 1990). It was formerly present in the Apennine mountains of Emilia Romagna, but its occurrence there could not be confirmed recently (Maggioni and Alessandrini, 2019). It reappears further east in the Republic of San Marino near the Italian Adriatic coast and then in Ancona and at Monte Conero (Maggioni and Alessandrini, 2019). On the Tyrrhenian side of Italy, it grows on the Pontine Islands (Anzalone *et al*, 2010). Punctual observations have been reported also further south in Campania, Basilicata and Calabria (Bartolucci *et al*, 2018), however, current presence and actual distribution in southern Italy would require a thorough investigation.

Collections documented in EURISCO with at least approximately known and reliable collecting locations comprise 57 accessions. These originate from Gerona province in Spain (16), south France (26) and Italy (15, of which 9 from Liguria, 4 from Tuscany and 2

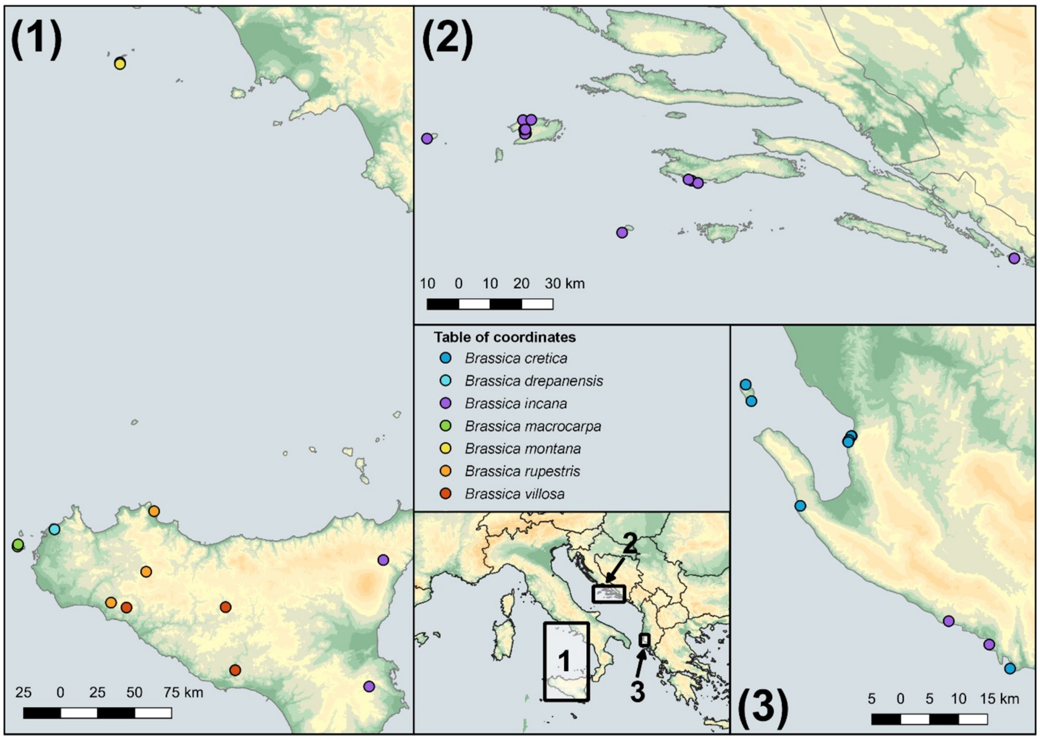

Figure 1. Collecting sites in Italy (1), Croatia (2) and Albania (3). Map created with QGIS v3.28.11-Firenze, (CC BY-SA 3.0)

from Marche regions). Gaps in *ex situ* collections are especially evident for central and south Italy.

The recent account that one sample from Ponza was highly resistant to *Xanthomonas campestris* (Sheng *et al*, 2020) raised increased interest and the need to secure the conservation of populations from the Pontine islands.

Brassica rupestris Raf.

[Synonym: *Brassica oleracea* L. subsp. *rupestris* (Raf.) Gladis & K. Hammer]

It is distributed mainly in north and west Sicily (Snogerup *et al*, 1990) and also found in south-eastern Calabria (Hammer *et al*, 1987). More recently, few sites have been identified also in eastern Sicily (Branca pers. comm.) and southwestern Calabria (Maggioni, pers. comm.). According to EURISCO, European genebanks only hold 22 accessions from western Sicily, conserved in Spain (13), Germany (4), the United Kingdom (3) and Italy (2). The genetic diversity of a few localized populations from the provinces of Palermo and Reggio Calabria has been studied extensively (Maggioni *et al*, 2014). Otherwise, a thorough analysis of the geographic distribution and diversity of this species is lacking and serious gaps exist in the *ex situ* European collections.

Brassica villosa Biv.

[Synonyms: *Brassica villosa* Biv. subsp. *tinei* (Lojac.) Raimondo & Mazzola; *Brassica villosa* Biv. subsp. *bivoniana* (Mazzola & Raimondo) Raimondo & Mazzola; *Brassica villosa* Biv. subsp. *villosa*]

B. villosa is endemic to the northwestern and central part of Sicily, in a few cases coastal, otherwise mostly found at inland cliffs localities (Snogerup *et al*, 1990). Various taxonomic treatments exist for this taxon, which is structured into various subspecies according to Malfa *et al* (2020), also including *B. drepanensis* as a subspecies of *B. villosa*. According to EURISCO, only 26 accessions of *B. villosa* are conserved in European genebanks, excluding those samples referring to *B. drepanensis*. These are conserved in the United Kingdom (11), Spain (7), Germany (5), Italy (1), the Netherlands (1) and Sweden (1). In some cases, the location of the collecting is not indicated, or it is placed in eastern Sicily, which makes the taxonomic attribution doubtful. Based on these data, the overall European *ex situ* collection of *B. villosa* is probably underrepresented. A thorough taxonomic revision including molecular markers and a better definition of the actual distribution range of *B. villosa* would also be beneficial and enable defining gaps in the existing collections.

Collecting missions – Methods and results

Collecting missions were carried out by project partner teams in Albania, Croatia and Italy (Lazio and Sicily). The composition of the teams, preparatory steps, itineraries and results are summarized below for each mission. All the collection sites are shown on a map of Europe in Figure 1. Information on collected accessions is summarized in Table 1.

Table 1. Summary of surveyed populations and collected accessions. Accessibility: A, The entire population or up to 80% of the plants are accessible; B, Between 20% and 80%; C, Less than 20% accessible and D, The entire population is inaccessible unless special equipment is used.

Taxon	FAO Country Code	Locality	Collecting date	Collecting numbers	GPS coordinates	Receiving Genebank (FAO Code)	Genebank accession numbers	Collected material	Status of the population
***Brassica cretica* Lam.**	ALB	Vlore, Tuneli, Depo ujit	Jul 2020	SJ;NH;LF019, SJ;NH;LF020, SJ;NH;LF021	40.421485; 19.488230	ALB026	AGB4451; AGB4452; AGB4453;	Seed	Small population; Accessibility: B
	ALB	Vlore, Uji Ftohtë, Moli ujit	Jul 2020	SJ;NH;LF022, SJ;NH;LF023	40.421293; 19.487295	ALB026	AGB4454; AGB4455	Seed	Small population; Accessibility: C
	ALB	Vlore/Bar-Restaurant Kala	Jul 2020	SJ;NH;LF024, SJ;NH;LF025, SJ;NH;LF026, SJ;NH;LF027	40.412923; 19.480489	ALB026	AGB4456; AGB4457; AGB4458; AGB4459	Seed	Few individuals; Accessibility: C
	ALB	Vlore/Resort Marina Bay	Jul 2020	SJ;NH;LF029, SJ;NH;LF030	40.415465; 19.482669	ALB026	AGB4461; AGB4462	Seed	Few individuals; Accessibility: B; endangered by constructions
	ALB	Radhimë	Jul 2020	SJ;NH;LF028	40.412221; 19.481160	ALB026	AGB4460	Seed	Few individuals; Accessibility: B
	ALB	Sazan Island – South side	Jul 2021	SJ;NH;LF039, SJ;NH;LF040, SJ;NH;LF041	40.480726; 19.286550	ALB026	AGB4471; AGB4472; AGB4473;	Seed	Large population; Accessibility: B
	ALB	Sazan Island – North side	Jul 2021	SJ;NH;LF042, SJ;NH;LF043	40.506662; 19.276134	ALB026	AGB4474; AGB4475;	Seed	Large population; Accessibility: B
	ALB	Alican – Karabuni	Jul 2021		40.315463; 19.379214			Not collected	Relatively large population; Accessibility: D
	ALB	Palermo Rock	Jul 2021		40.051141; 19.792803			Not collected	Small population; Accessibility: B
***Brassica drepanensis* (Caruel) Damanti**	ITA	Erice	25 Jul 2021		38.03505556; 12.59147222	ITA331	UNICT 5288	Seed	Abundant; Accessibility: B

Continued on next page

Table 1 continued

Taxon	FAO Country Code	Locality	Collecting date	Collecting numbers	GPS coordinates	Receiving Genebank (FAO Code)	Genebank accession numbers	Collected material	Status of the population
Brassica incana **Ten.**	ALB	Himarë /Potam beach	Jul 2020	SJ;NH;LF032, SJ;NH;LF033, SJ;NH;LF034, SJ;NH;LF035, SJ;NH;LF036	40.089685; 19.752504 40.088730; 19.750805	ALB026	AGB4464; AGB4465; AGB4466; AGB4467; AGB4468	Seed	Relatively large population; Accessibility: A/B
	ALB	Gijpe			40.128361; 19.672164			Not collected	Small population; Accessibility: B
	HRV	Sušac	Jul 2022	IPT521	42.752716, 16.490663	HRV050	IPT521	Seed	<20 plants; Accessibility: B
	HRV	Kosor	Jul 2022	IPT518	42.901346, 16.761636	HRV050	IPT518	Seed	20 plants; Accessibility: A
	HRV	Stupa	Jul 2022	IPT522	42.894560; 16.786568	HRV050	IPT522	Seed	10 plants; Accessibility: A
	HRV	Obljak	Jul 2022	IPT520	42.904644, 16.749480	HRV050	IPT520	Seed	20 plants; Accessibility: A
	HRV	Koločep	Oct 2021	IPT517	42.668715, 18.014589	HRV050	IPT517	Seed	30 (–40) plants; Accessibility: B
	HRV	Svetac	May 2023	IPT 618	43.019725, 15.728069	HRV050	IPT 618	Seeds and small plants	20 plants; Accessibility: B
	HRV	Vis (Oključina)	May 2023	IPT 619	43.074649, 16.102745	HRV050	IPT 619	Seeds and small plants	15 plants; Accessibility: B
	HRV	Vis (Gradac)	May 2023	IPT 620	43.075623, 16.134977	HRV050	IPT 620	Seeds and small plants	10 plants; Accessibility: B
	HRV	Vis (St. Duh)	May 2023		43.036415, 16.114780			Not collected	3 plants; Accessibility: B
	HRV	Vis (hiking trail from St. Duh church to St. Nikola church)	May 2023	IPT 621	43.034960, 16.110665	HRV050	IPT 621	Seeds	5 plants; Accessibility: B
	HRV	Vis (Crvene stijene)	May 2023	IPT 622	43.047342, 16.107393	HRV050	IPT 622	Seeds and small plants	20–30 plants; Accessibility: A

Continued on next page

Table 1 continued

Taxon	**FAO Country Code**	**Locality**	**Collecting date**	**Collecting numbers**	**GPS coordinates**	**Receiving Genebank (FAO Code)**	**Genebank accession numbers**	**Collected material**	**Status of the population**
***Brassica incana* Ten.**	HRV	Vis (St. Mihovil church)	May 2023	IPT 623	43.048160, 16.112421	HRV050	IPT 623	Seeds	5 plants; Accessibility: B
	ITA	Sortino	9 June 2022		37.133350, 15.031676	ITA331	UNICT 5310	Shoots for cutting	Very few individuals; Accessibility: C
	ITA	Francavilla	20 Jul 2021		37.90494444; 15.12491667	ITA331	UNICT 5311	Seed, shoots for cutting and small plants	Abundant; Accessibility: C
***Brassica juncea* (L.) Czern.**	ALB	Himarë	June 2020	SJ;NH;LF037, SJ;NH;LF038	40.101337; 19.741662	ALB026	AGB4469; AGB4470	Seed	
***Brassica macrocarpa* Guss.**	ITA	Favignana	25 Jul 2021		37.92108333; 12.31330556	ITA331	UNICT 5289	Seed	Abundant; Accessibility: C
	ITA	Favignana	25 Jul 2021		37.93400000; 12.31694444	ITA331	UNICT 5308	Seed	Abundant; Accessibility: C
	ITA	Marettimo	25 Jul 2021		37.94880556; 12.08336111	ITA331	UNICT 5309	Not collected	Very few individuals; Accessibility: D
***Brassica montana* Pourr.**	ITA	Ponza	June 2021	LM21-01; LM21-02	40.888889; 12.953056	ITA331 and Orto Botanico Roma	UNICT 2306 UNICT 5316	Seed	Very few individuals; Accessibility: A; endangered by landslides
	ITA	Ponza	June 2021		40.879751; 12.953690			Not collected	Very few individuals; Accessibility: D

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Table 1 continued

Taxon	**FAO Country Code**	**Locality**	**Collecting date**	**Collecting numbers**	**GPS coordinates**	**Receiving Genebank (FAO Code)**	**Genebank accession numbers**	**Collected material**	**Status of the population**
***Brassica rupestris* Raf.**	ITA	Corleone	25 May 2021		37.798463, 13.307139	ITA331	UNICT 5383 UNICT 5326 UNICT 5327 UNICT 5328	Seed	Abundant; Accessibility: B
	ITA	Palermo	25 May 2021		38.16830556; 13.35211111	ITA331	UNICT 5290	Seed	Abundant; Accessibility: B
***Brassica rupestris* subsp. *tardarae* (Ilardi & al.) Raimondo**	ITA	Sambuca di Sicilia	25 May 2021		37.615472; 13.053333	ITA331	UNICT 5286	Seed	Less than 100 individuals; Accessibility: C
***Brassica villosa* Biv.**	ITA	Caltabellotta	25 May 2021		37.57669444; 13.16616667	ITA331	UNICT from 5329 to 5335	Seed	Few individuals; Accessibility: C
***Brassica villosa* subsp. *tineoi* (Lojac.) Raimondo & Mazzola**	ITA	Butera	23 Mar 2022		37.21597222; 14.00886111	ITA331	UNICT 5291	Shoots for cuttings	Abundant; Accessibility: C
	ITA	Marianopoli	23 Mar 2022		37.597965; 13.923801	ITA331	UNICT 5292	Shoots for cuttings	Very few individuals; Accessibility: B
	ITA	Butera	20 Oct 2022		37.21577778; 14.01016667	ITA331	UNICT 5312	Seed	Endangered; Accessibility: C

Albania

Under the leadership of Sokrat Jani, the Institute of Plant Genetic Resources, Agricultural University of Tirana, organized a mission targeting the exploration, identification and collection of seed samples of wild relatives of *Brassica* (*B. cretica* Lam. and *B. incana* Ten.) in Ionian coast area and Sazan Island.

The collecting team was composed of Sokrat Jani, Institute of Plant Genetic Resources, Tirana, Nexhip Hysolakoj, Regional Agency of Protected Areas of Vlora and Lavdosh Ferruni, Organic Agriculture Association of Albania.

The travel target areas were the Ionian coast of Vlora and Himarë, and Sazan Island, a small uninhabited island with an area of 5.7km^2 and a coastline of about 21km, at the Gulf of Vlora. During the years 2019–2021, nine field missions were undertaken, three each year respectively around 2–20 April, which coincides with the blooming phase; 10–20 May, which coincides with the fruiting period and 20 June–10 July, which coincides with the seed ripening period. To reach Sazan Island, the team used the boat of the Regional Agency of Protected Areas of Vlora. There are no regular roads for driving on the island, therefore the group explored the area on foot.

Information available about the sites to visit was based on literature, herbarium specimens and personal knowledge. The first documentation about the presence of two maritime *Brassica* species in the Ionian coastal region of Albania was published in Flora of Albania for two localities, Spile (Himarë) for *B. incana*, and Vlora for *B. oleracea* subsp. *oleracea* (Paparisto and Qosja, 1976). A brief description of *B. incana* at Himarë is given in Flore de l'Albanie (Qosja *et al*, 1996). Based on the material collected during their field trips to Albania from 2008 to 2010, Barina *et al* (2011) presented some confirmations for chasmophytic *Brassica* species in three localities, of which two are the same as those of Paparisto and Qosja (1976). In Spile Bay (Potam beach) at Himarë, they confirmed the occurrence of *B. incana*, while *B. cretica* was observed near Vlora, about 4km north of the village of Radhimë, and in the Porto Palermo Peninsula. Subsequently, during a field trip on 4 April 2019 a group consisting of Lorenzo Maggioni (ECPGR Secretariat), Eva Thörn (ECPGR Executive Committee), Imke Thormann (Federal Office of Agriculture and Food, Germany), Fetah Elezi and Sokrat Jani from Agricultural University of Tirana, surveyed the presence of a small population of 12 reproductive plants and some vegetative individuals of *B. cretica* in the front part of the cliff, on both sides of the coast road tunnel at Uje i Ftohtë, as well as a small population on the southwest side of the coast, near Bar Kalaja. Further south, by the Potam beach (Himarë), at the base of the cliff, on its northwestern side, a medium-sized population of about 30 reproductive plants and some vegetative individuals of *B. incana* was surveyed by the same group. Both species were then in the flowering phase.

These expeditions aimed at gathering information, exploring more localities for possible other population sites, finding and identifying species, assessing the situation, evaluating vegetation status and habitats, and collecting seed material for conservation and use.

Samples were identified in the field, based on morphological characters. During the ripening period, seed samples were collected from most populations. This was done according to a collection protocol trying to capture the maximum variability of the population (from as many plants as possible), while avoiding harm to the populations themselves (harvesting only a small part of the seeds produced by any plant). Mature siliquas were collected from individual plants and kept separately in cotton bags. The size of the populations was estimated, distinguishing vegetative and reproductive individuals, as well as human and livestock accessibility. GPS coordinates were taken with a GPSMAP 60CSx. After return from the field, the collected seed samples, after being processed, were included in the Albanian Genebank, Tirana, for long-term storage. As the terrain was difficult, the working group was supported with the necessary tools and equipment, such as cars, boats, drones, professional cameras, etc., by the Regional Agency of Protected Areas, Vlora.

Results

Out of 23 sites visited with a suitable habitat, wild brassicas were observed in 13 of them. The results obtained from the exploration are described below.

Ionian coast area of Vlora

The presence of *B. cretica* was observed in five sites in the Vlora Bay area (two sites near the tunnel area in Uje i Ftohtë, and respectively one each in Marina Bay Resort, Bar-Restaurant-Kala, and Radhimë, part of Kala road) and one on the Karaburun peninsula (in the western part, from Alicani to Bristani Bay):

Tunnel area of Uje i Ftohtë, in front of the cliff, northwest side: Two populations of *B. cretica* were identified there, respectively to the left and right of the tunnel entrance, called Depo ujit and Moli ujit, with 12 and eight reproductive plants, respectively, and very few vegetative individuals (new plants). These sites were respectively rated average and slightly accessible by humans and livestock. Seed was collected from three and two plants, respectively, separately in cotton bags.

Bar-Restaurant Kala area, south side: the site was first surveyed in 2019, and it was surprising in 2020 to note the disappearance of a small population of *B. cretica* from every accessible site along the base of the cliff, due to mowing of the area by resort gardeners. Not a single plant remained on the cliff, except at the bottom of it, in the inaccessible part, where only ten reproductive plants and very few young plants could be counted. Seeds were collected in 2020 from four plants, separately in cotton bags, with the help of two young climbers.

Radhimë, part of Kala road, west side: It seemed to be a newly established population, with 18 small reproductive plants and very few mature siliquas and many new plants, possibly formed by migrations from a now extinct *B. cretica* population of Jonufra (4km north of Radhimë), which was an old population documented by Paparisto and Qosja (1976) and later confirmed by Barina *et al* (2011). Seed was collected from only one plant.

Resort Marina Bay area, north side: At this site, two populations of *B. cretica* were located very close to each other in the same area and only 200–300m away from each other (Marina Bay and Resort Gjyla), with overall 12 reproductive plants and very few young plants; these were moderately accessible to humans and endangered of disappearance due to constructions in the tourist resort. Seed was collected from the two matured plants.

Karaburuni peninsula, west side/Alican – Brisani Bay: A site was explored with an area of 4,678m^2 and a relatively large population of *B. cretica*, with a density of 1–2 reproductive plants/100m^2. The first individual plants were identified in the Alican site, on vertical rocks, an area isolated and inaccessible to humans or livestock. Its extent, with few individual plants, was linear to the coast of the entire Gulf of Brisan. No seeds were collected, as the entire population was located on steep cliff tops and therefore inaccessible unless special equipment is used.

Skele neighbourhood of Vlora: In addition to the target species (*B. cretica* and *B. incana*), a small population of eight to ten plants initially identified as *B. rupestris* Raf. was found in a home garden in the coastal neighbourhood Skele of the city of Vlora. From the conversation with the owner of the home garden, it was learned that that population may have originated from a maritime limestone rock area about 7–8km away, called Kuzumbaba, where he had taken soil five years before to use for the home garden; but with the soil, it seems, the seeds also came. However, following a survey of the described area by our team, no plants of *B. rupestris* could be found. Seed was collected from home garden plants and sent to the Albanian Genebank, Tirana. After closer observation, these plants more probably belong to a domesticated type of leafy kale.

Ionian coast area of Himarë

Three sites were found with a good presence of *B. incana*, respectively in Potam, Gjipe bay and possibly *B. cretica* in the Porto Palermo peninsula.

Potam beach: In the southern part of the bay of Spile (Himarë), in a rocky strip with a length of about 2–3km, on maritime limestone rocks, the presence of a relatively large population of *B. incana* was reconfirmed, linear to the coast, with 28 reproductive plants and many new vegetative individuals, which was previously observed and documented by Paparisto and Qosja (1976) and later confirmed by Barina *et al* (2011); every part of it was accessible along the base of the cliff. Seed was collected from five plants.

Himarë Seaport, south side: Besides the two target species (*B. cretica* and *B. incana*), in a home garden, near the Himarë Seaport, a small population of four plants of *B. juncea* (L.) Czern, apparently spontaneously grown was explored. Seed was collected from two plants. Unfortunately, our monitoring in the spring of 2022 revealed that this population had disappeared.

Other localities: other parts of the coast of Himarë were surveyed, north and south of the bay of Spile (Himarë), but new *Brassica* sites were found only in the Gjipe bay (near Gjipe canyon), about 15km north of Himarë and on the peninsula of Porto Palermo, about 9km south of Himarë, at a site known as The Rock of Porto Palermo. It was surprising to notice the disappearance of the *B. cretica* population in the Cave site of Porto Palermo, which was described in 2010 by Barina *et al* (2011). However, a new and smaller population was found, about 1km northwest, in a segment with straight and steep rocks, in the place known as The Rock of Porto Palermo. It seems that livestock may have influenced the migration of *Brassica* vegetation from one site to the other. We estimated that in Gjipe bay and The Rock of Porto Palermo, near the Falling Rocks Beach, 18 and 30 reproductive plants of *B. incana* and *B. cretica* (to be verified) were growing, and some vegetative individuals, but it was not possible to collect seeds from either site.

Sazan Island area, east and northeast side

The large presence of *B. cretica* on the island was surprising, mainly concentrated in two sites, the South Cape and the North Cape. The largest area with more reproductive plants and young plants was located in the south, in the Gully of Jehnem site, where the density was 30–40 reproductive plants/100m^2. Seeds were collected from the two sites, respectively from three and two plants.

Croatia

Under the leadership of Nenad Jasprica, Institute for Marine and Coastal Research, University of Dubrovnik, and Smiljana Goreta Ban, Institute of Agriculture and Tourism (IPTPO), Poreč, two expedition missions were organized, targeting several wild *Brassica* species in the southern Croatian islands and islets. The goals of the expeditions were to check the status of the *Brassica* species populations, to collect seeds or young plants from previously known sites and to estimate the possible losses that happened during previous periods.

Apart from N. Jasprica and S. G. Ban, the collecting team also consisted of Nina Išić and Dean Ban from the Institute of Agriculture and Tourism, Poreč, Branka Salopek Sondi from the Ruer Bošković Institute, Zagreb, and Mirta Tkalec and Nataša Bauer from the Faculty of Science, University of Zagreb.

Preliminary information on the distribution, habitat, ecology and phenology of insular brassicas in Croatia was obtained from the Flora Croatica Database (Nikolić (2005) and onwards). This database contained data from the literature, field observations and references to herbarium specimens, and allowed further detailed study with the aim of completing knowledge on the subject.

The first expedition started on 5 July 2022, travelling to Split and taking the ferry to Vela Luka located on the Korčula Island. The exploration of the island's localities took place over two days, 6-7 July 2022. On 6 July, the team visited the islands Sušac (Lastovo Islands Nature Park), and islets Obljak, Kosor and Stupa (Korčula Archipelago) by speedboat, and on 7 July the collecting team visited two previously targeted localities (the pebble beach of Vaja and the Bay of Samograd) near the Račišće village on the northern coastline of Korčula Island. In addition, the island of Koločep near the city of Dubrovnik was also previously visited on 7 July 2020, 1 October 2021 as well as on 15 July 2022. During the first expedition, mature siliques were collected from four localities: the island of Sušac, and the islets of Obljak, Kosor and Stupa. GPS coordinates were determined with an Android device. Seeds from one plant were collected on Sušac Island, while from each of the other three islands, seeds were collected from approximately ten plants. Siliquas were kept in paper bags. The seeds were counted with Contador optical seed counter and weighted.

The second expedition took place between 22 and 26 May 2023. The expedition included localities previously mentioned in the literature on the island of Vis and the island of Svetac (locally named St. Andrija) (Nikolić (2005) and onwards). The exploration of the two islands took place over two days, 23-24 May 2023. On 23 May, the collecting team visited the Svetac Island by speedboat, followed by several localities on the Vis Island, mainly north-facing cliffs and small coves. On 24 May, the third day of the expedition, the team visited previously mentioned inland localities of Vis, the first one being beneath St. Duh church. Next, the team followed a hiking trail from St. Duh church to St. Nikola church towards Komiža city where the presence of a *B. incana* population was previously recorded. The team then travelled to a location known as Crvene stijene (red rocks), a sport climbing area, where an abundant *B. incana* population was found. The next location was near St. Mihovil church, while the presence of a military zone prohibited the members from visiting the location above Podhumlje. The expedition members spent the fourth day travelling home. GPS coordinates were determined with an Android device. During the second expedition, unripe green siliquas were collected from six localities: the island of Svetac, two coastal localities on the island of Vis (Oključina and Gradac) and three inland localities on the island of Vis (hiking trail near St. Duh church, Crvene stijene and St. Mihovil). Young plants for vegetative propagation were collected from the island of Svetac, two coastal localities on the island of Vis (Oključina and Gradac) and one inland locality on the island of Vis (Crvene stijene). Siliquas were kept in paper bags.

Results

Koločep Island

The population of *B. incana* (locally named *B. mollis*) was quite numerous on the island (more than 50 individuals). It mainly inhabited the vertical sea cliffs oriented towards SW-SE, reaching up to 60m a.s.l. On the vertical profile of the cliffs, it colonized the halotolerant vegetation of the saline areas up to the bushy vegetation and Aleppo pine near the tops of the cliffs.

Sušac Island, southwest side

The population of *B. incana* (locally named *B. cazzae*) was reduced to a single reproductive plant seen by the expedition team near the Sušac lighthouse. Since the plant was on an inaccessible cliff, no seed was collected from this plant. Vegetative individuals were found on the island in the halophilous vegetation of the salt-sprayed rocky cliffs at 90–110m a.s.l., but also in the maquis on the cliff top.

Sušac Island, northwest side

One reproductive plant and several young ones were found near the trail leading to the Sušac lighthouse. Mature siliquas were collected in a paper bag and one entire plant was taken for vegetative propagation.

Obljak, Kosor and Stupa islets

On each of these three islets, the team found more than 20 individuals of *B. incana*. The mature siliquas of about ten plants from each islet were collected in paper bags. On Obljak and Kosor, the plants were found on the entire surface of the islets. Most of the *Brassica* population on Stupa was on the northern slope of the islet. The species occured mainly in the herbaceous vegetation of the salt-sprayed low rocky coasts, but also in the maquis in the upper belt, which is only weakly influenced by the sea aerosol.

Two small bays near the village of Račišće, Korčula Island

The team found no individuals of *B. incana* at these two previously known sites, a result of habitat loss due to anthropogenic impacts. These sites consist of gravel shorelines exposed to wave action, resulting in a thicker or shallower layer of pebbles at the surface. Nitrogen-rich humus often forms beneath the surface.

Svetac Island

The *B. incana* population on the west coast of Svetac Island consisted of about 20 plants, a good portion of them in reproductive stage. The team collected unripe, green siliquas from three mature plants and two young plants. The siliquas were collected in paper bags.

Vis Island

In the locality Oključina the team found about 15 plants, several of them in reproductive stage. The team collected unripe, green siliquas from three plants and took two young plants.

In Gradac cove the team found around ten *B. incana* plants and one plant morphologically similar but with smooth, hairless leaves and stems. Several *B. incana* plants were in the reproductive stage and siliquas were taken from two plants along with two entire young plants. Seeds were taken also from the morphologically similar species, and one young plant was collected just beneath the reproductive one.

In the inland location of St. Duh the team only found three young *B. incana* plants in vegetative stage. Due to the lack of siliquas and a very small number of individuals, no siliquas or plants were taken.

On the hiking trail from St. Duh church to St. Nikola church in Komiža, the team found five *B. incana* plants. Several plants were in reproductive stage, so the team collected a small portion of unripe green siliquas from two plants. Because it was only a small population, no young plants were taken.

The locality Crvene stijene hosted the most abundant population in the Vis Island, counting more than 20 *B. incana* plants, several of them in reproductive stage. The team collected a small portion of green siliquas from five plants and two young plants.

Around five plants were found near St. Mihovil church with only two plants in reproductive stage. The team collected a small portion of green siliquas from the two plants and no young plants were collected.

Italy

Island of Ponza, Lazio, Italy

Lorenzo Maggioni, ECPGR Secretariat, Rome, carried a mission to the island of Ponza on behalf of the University of Catania (UNICT), targeting *B. montana*. Travelling from Rome to Formia and then taking the ferry to Ponza on 13 June 2021, the exploration of the island took place over two days, 14 and 15 June 2021 by foot and using the public bus. Selected dates were suitable to find mature siliquas, based on previous experience. Information available about the sites to visit was based on literature, herbarium specimens and personal knowledge. In their map of vegetation series of Ponza, Stanisci *et al* (2005) indicated the presence of *B. montana* as a companion species at localities Monte Guardia and Faro. Anzalone *et al* (2010) indicate the presence of *B. montana* in the Pontine Islands. Herbarium specimens in the Anzalone Herbarium in Rome [RO-HA] were collected from localities Monte Guardia in 1966 and Forna in 1974. During a previous survey in March 2004, from the trail midway up to the top of Monte Guardia, northwest side, Maggioni could count more than 200 flowering plants in a gully facing the famous cliff and beach of Chiaia di Luna. This gully was subsequently subject to a rock collapse which probably destroyed a large part of this population and the trail became almost inaccessible. Other individual plants could also be observed on formerly cultivated abandoned terraces, closer to the top of the mountain. In July 2005, at the southwest side of Monte Guardia, Maggioni surveyed the presence of a relatively large population at the base of the cliff, all along the trail reaching the lighthouse at Punta della Guardia.

The objective of the present survey was to collect seeds from sites already known and explore the rest of the island for possible other population sites. Mature siliquas were collected from individual plants and kept in separate paper bags. The size of the population was estimated, distinguishing vegetative and reproductive individuals. GPS coordinates were taken with a cellular phone. After return from the field, siliquas were manually threshed in paper trays and the number of seeds was approximately counted (data not shown).

Results

Monte Guardia, northwest side: it was confirmed that the relatively large population observed in 2004 had almost disappeared and only two reproductive plants and very few vegetative individuals could be found on or along the trail. Seed was collected from the two plants [collecting numbers LM21-01 and LM21-02]. Paper envelopes containing respectively ca. 200 and 100 seeds were sent to UNICT. One envelope with ca. 50 seeds of LM21-01 was also sent to the Botanic Garden of the University La Sapienza in Rome, Italy (care of Giuseppe Fabrini).

Monte Guardia southeast side: it was surprising to note the disappearance of *B. montana* from any accessible site along the base of the cliff. No plants could be identified on the cliff either, except at the very end of the trail, on the small promontory Punta della Guardia, hosting the lighthouse. Here, less than ten vegetative plants could be observed in inaccessible positions. In recent years, this area has been subject to landslides that have disturbed the base of the cliff. Also, the abundant presence of *Dittrichia viscosa* (L.) Greuter, widely colonizing the area, might have become a competitor to *B. montana*.

Other localities: other parts of the island were surveyed (descent to Punta del Fieno; Piana d'Incenso up to Punta Incenso; Fortino del Papa and surroundings; and descent to Scogli della Cantina) but no new sites were found. The locality Forna was not thoroughly surveyed and might deserve a more careful check, considering that one herbarium sample was collected at this locality in 1974 by Anzalone, but with no detailed site indications.

Sicily

Under the leadership of Ferdinando Branca, UNICT, Italy, organized three missions targeting several wild *Brassica* species growing in Sicily. The UNICT team, represented also by the PhD students Maria Concetta Di Bella, Simone Treccarichi, Donata Arena and Giulio Flavio Rizzo, collected several samples in natural areas during three expeditions, also evaluating the status of the populations. In total, six expeditions were carried out, travelling by car from Catania, between May 2021 and October 2022, targeting the provinces of Palermo and Agrigento (I), Erice and Egadi Islands (II), Butera and Marianopoli, province of Caltanissetta (III), Sortino, province of Siracusa (IV), Francavilla di Sicilia, province of Messina (V) and Butera and mount Muculufa, province of Caltanissetta (VI).

The aim of the expeditions was to increase the availability of seeds for the biomorphological, biochemical and genetic characterization of these populations which were not available in genebanks. Based on previous personal knowledge and local information, the team explored the above-mentioned sites to observe the status of the populations (distribution and estimated number of plants) and seed samples were collected based on the biological status of the plants. Information regarding the collecting sites was obtained through Google Earth and by interviews with local residents.

The collecting methodology encompassed either harvesting of siliquas from individual plants, which were kept separate in the case of *B. rupestris* of Corleone and *B. villosa* of Caltabellotta, or bulked from several plants for all other populations for which seeds were collected. Additionally, vegetative cuttings were also collected. Siliqua samples were stored in paper bags to avoid mould formation. Subsequently, siliquas were cleaned at the University of Catania, specifically in the Lab of Biotechnology of Vegetable Crops belonging to Dipartimento di Agricoltura, Alimentazione e Ambiente (Di3A). As concerns the cutting method, it consisted of oblique cutting carried out with professional pruning shears. Fresh material was stored in plastic bags to maintain high moisture conditions.

Results

The first expedition (I) was carried out at the end of May 2021 and five different accessions were collected: two accessions of *B. rupestris* from Corleone and Monte Pellegrino (Palermo province), one *B. rupestris* subsp. *tardarae* (Ilardi & al.) Raimondo at Gole della Tardara, near Sambuca di Sicilia (Agrigento province), one accession of *B. villosa* from Caltabellotta (Agrigento province). The first site is located south of Corleone on the Rocca cliffs, where an abundant population of *B. rupestris* is widespread. With regard to Mt. Pellegrino, an important population of *B. rupestris* was found around the cliffs of the Monastery of Santa Rosalia in Palermo, which is the type site for *B. rupestris*. The population of the subsp. *tardarae* was not abundant or much diversified in terms of plant age, whereas the population of *B. villosa* identified around Pizzo Telegrafo and the surrounding areas near Caltabellotta was abundant.

During the second expedition (II) at the end of July 2021 in the province of Trapani, three different accessions were collected. Two accessions of *B. macrocarpa* were collected on the islands of Favignana, in two different stations. Of these two populations, the more abundant was located on Monte Santa Caterina, spreading from the top of the mountain down to the sea coast. On the other hand, on the island of Marettimo, a few individuals were noted in cliffs that were not well accessible around Punta Bassana. Another population in Marettimo was located on the top of Monte Falcone, but this was not visited. Finally, one accession of *B. drepanensis* was collected on Mount Erice.

The third expedition (III) was carried out in March 2022 at Mount Muculufa, near Butera and Marianopoli, Caltanissetta province, monitoring two populations of *B. villosa* subsp. *tineoi* (Lojac.) Raimondo & Mazzola and collecting shoots for establishing rooted cuttings. Of the two identified populations, the former was abundant whereas the latter was represented by few individuals.

The fourth expedition (IV) was organized at Sortino, Siracusa province, in June 2022, monitoring the populations of *B. incana* and collecting shoots for establishing rooted cuttings. The fifth expedition (V) was organized at Francavilla di Sicilia, Messina province, in July 2022, collecting *B. incana* seeds and cuttings for rooting. Finally, the sixth and last expedition (VI) was organized near Ravanusa exploring Monte Muculufa, Caltanissetta province, in October 2022, where a population of *B. villosa* was found in a chalk quarry, endangered by human activities (caves for salt extraction) and fires.

Conclusion

Although the distribution of the wild populations belonging to the gene pool $2n = 18$ of *B. oleracea* has been intensely investigated, starting in the 1970s, a full overview of all the existing populations is far from complete. Even for well-known populations, major gaps remain in the available documentation of the European genebanks and access to documented populations is often uncertain or not guaranteed, owing to limited seed availability, difficult and expensive multiplication or other limitations. The ECPGR-funded EUBRASWILD project enabled collecting activities focused on some of the less documented locations within the distribution range of these wild brassicas. Each mission was carried out by local teams within their country, with the intention to eventually make both information and genetic material publicly available. As indicated in Table 1, all accessions have been deposited in the respective genebanks with an assigned accession number. Requests from potential users can be immediately honoured in case of sufficient seed availability. The missions carried out in Albania were for the first time specifically dedicated to these taxa and allowed to describe with a good degree of accuracy several populations of *B. incana* and *B. cretica*, including some locations that had never been described before (*B. cretica* in the Sazan Island and Karaburun peninsula and *B. incana* in the Gjipe bay). In other cases, habitat loss due to direct or indirect human intervention is affecting the size or the existence of some populations. For the first time, seed samples were collected for long-term conservation, deposited in the Albanian Genebank and publicly documented in the EURISCO catalogue. The Croatian missions were successful in monitoring already known populations of *B. incana* and collecting seed for long-term conservation and further characterization. The possible disappearance of two populations on the island of Korčula due to habitat loss was an unpleasant observation. The collected populations will be documented in the EURISCO catalogue.

The expeditions carried out in Italy also enabled monitoring of existing populations and discovering previously unknown sites, such as the case of *B. villosa* subsp. *tineoi* near Butera and to monitor the populations of the already-known sites. A few sites were observed, where the populations are endangered by human activities. On the island of Ponza, it was significant to notice the severe reduction, close to disappearance, of *B. montana*, possibly due to a change of habitat determined by natural causes. The few seeds collected may deserve careful attention if it is confirmed that resistance to *Xanthomonas* has been found in a plant from Ponza. The very poor documentation in EURISCO of the populations conserved in Italy is improving as a result of this activity.

Overall, the activities carried out in this project have added important pieces of information on the status and trends of a few Mediterranean wild *Brassica* populations, have increased the level of collaboration across countries and obtained material which will be useful for further collaboration regarding its characterization and possible use. Given the importance and immense diversity potential of the gene pool of *B. oleracea*, which is a unique resource of the European and Mediterranean region, it is hoped that more systematic investigations with larger breadth and funding can be planned in the future (including to sort out a complicated taxonomy which is currently only based on very unstable and unreliable morphological characters). Gathered information should also be useful to raise the awareness of national and local authorities about the need to take action for *in situ* conservation of the most endangered populations.

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Author contributions

FB, LM, SGB and SJ contributed to the overall conception and planning of the collecting missions. These were carried out by SJ and colleagues in Albania, SGB, NJ, NI and colleagues in Croatia, LM in Italy (Ponza), FB, ST and colleagues in Italy (Sicily). The first draft of the manuscript was written by LM and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of interest statement

The authors have no conflicts of interest to report.

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Genetic divergence study on growth, yield and quality traits in pink brinjal (*Solanum melongena* L.) in the subtropical plains of Jammu, India

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Abstract: In 2022, field investigation was conducted to examine the genetic diversity among 30 pink brinjal (eggplant, *Solanum melongena* L.) genotypes originating from India. Mahalanobis D^2 analysis was performed to analyze the data for eighteen growth, yield and quality characters. Among the traits examined, the total fruit yield per plant made the most significant contribution towards diversity. Thirty genotypes were meaningfully grouped into eight clusters. Cluster I, the largest, had eight genotypes, followed by Cluster II and Cluster IV. There was no direct correlation observed between the geographical distribution and genetic divergence. Among all clusters formed, Cluster VII exhibited maximum intra-cluster distance followed by Cluster VI. Observing the inter-cluster distances, the maximum divergence was noted between Cluster III and Cluster IV suggesting that genotypes within these clusters could serve as valuable parents for hybridization programmes aimed at producing highly heterotic hybrids and identifying transgressive segregants in the F_2 generation.

Keywords: Hybridization, Genetic diversity, intracluster distance, eggplant, *Solanum melongena* L, D2 analysis

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Introduction

Brinjal or eggplant, scientifically known as *Solanum melongena* L., with a chromosome number of 2n=2x=24, is a significant vegetable plant within the Solanaceae family. In India, the second largest producer worldwide (FAO, 2021), brinjal is mostly cultivated in West Bengal, Odisha, Gujarat, Bihar and Madhya Pradesh. Although it is a perennial by nature, it is predominantly grown and harvested as an annual crop for its young, unripe fruits, commonly utilized in a variety of cooked dishes. It is considered to be a rich source of abundant nutrients and is the complete set of minerals, vitamins, nutritional fibre, protein, and antioxidants, along with some phytochemicals like caffeic acid, chlorogenic acid (phenolic components) glucoside, delphinidin, and nasunin (flavonoids) that have scavenging activities (Noda *et al*, 2000; Bhaskar *et al*, 2015).

As per Zeven and Zhukovsky (1975), brinjal's primary origin is traced back to India, while China is considered a secondary centre of its origin. A large indigenous biodiversity exists in eggplant in the Indian subcontinent region, due to the place being its centre of origin, and there exists a great variation in plant type, stem colour, leaf size, leaf tip, midrib colour, fruit shape, fruit size, fruit colour, fruit yield, cooking quality, fruit quality, and tolerance to pests and diseases (Ullah *et al*, 2014).

Understanding the genetic diversity within breeding materials is crucial for plant breeders to effectively select parent plants for crossing schemes, which is a prerequisite in breeding programmes. Genetic diversity serves as important criterion for the selection of diverse parents who are expected to produce high hybrid

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vigour (Harrington, 1940). Crossing parents chosen for their genetic divergence, is likely to yield transgressive offspring, and subsequent selection can be applied in segregating generations. The Mahalanobis D^2 technique, as recommended by (Rao, 1952) is a recognized method based on multivariate analysis, offering a reliable measure of genetic diversity. The objective of this study was to investigate the genetic diversity among 30 diverse brinjal genotypes in the subtropic plains of Jammu, India to assist the breeder in identifying prospective parents that exhibit promising traits and possess genetic diversity to achieve the desired improvement.

One of the primary goals in brinjal breeding is achieving early flowering and early harvest, as these traits enable farmers to catch the early market, leading to higher returns. Plant spread, plant height and number of branches per plant are other important traits that directly impact on number of fruits and, consequently, the yield, leading to higher returns to farmers. Fruit length, fruit diameter and fruit weight are directly linked to fruit yield. Marketable fruit yield and unmarketable fruit yield based on fruits affected by biotic (disease-infected and pest-infested) and abiotic stresses (high and low temperature/freezing injury etc.) are also important traits in brinjal breeding. The ultimate goal of any crop improvement programme is to increase economic yield which is measured as fruit yield per plant and fruit yield per hectare. Germination percentage is a parameter used to measure seed viability, and crop stands in the field primarily depend on this percentage, ultimately determining the final yield. Seed vigour index is another important trait in crop improvement as seeds with a higher vigour index produce an early and uniform stand in the field. Ascorbic acid content in brinjal fruit is an important biochemical character associated with increased nutritive value of the fruits which promotes better retention of colour and flavour (Sasikumar, 1999). Total phenol content helps determine resistance against fruit and shoot borer incidence in brinjal. The higher the phenol content, the lower the incidence of fruit and shoot borer (Jat and Pareek, 2003; Shinde *et al*, 2009). In addition, higher phenolic levels influence antioxidant content and fruit culinary quality (Stommel *et al*, 2015). Keeping in view the importance of the above traits in brinjal breeding, these were taken into consideration in the present study.

Materials and methods

Thirty genotypes (Table 1) of pink brinjal grown across various regions of Jammu and Kashmir were gathered, tested and assessed at the Experimental Farm of the Division of Vegetable Science, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu. The material encompasses local landraces, advanced breeding lines and hybrids and is conserved at the institute. Seeds were sown in raised nursery beds of 3m × 1m size on 4August 2022 in lines spaced 5cm apart. Proper care was taken to water the beds and remove weeds for raising healthy seedlings. Seedlings were ready for transplanting after four and five weeks. Seedlings were transplanted on 2 September 2022 in a randomized complete block experimental design with three replications with a plot size of 3m × 1m and spacing of 90cm × 75cm. All the prescribed cultural practices were followed throughout the growth and development period of the crop to cultivate a healthy crop as given in Package and Practices for Vegetable Crops (Anonymous, 2020).

Five plants were selected from each plot to record observations on days to first flowering, days to first harvest, plant height (cm), number of branches per plant, plant spread (cm^2), number of fruits per plant, fruit length (cm), fruit diameter (cm), fruit weight (g), marketable fruit yield per plant (kg) unmarketable fruit yield per plant (kg), total yield per plant (kg), fruit yield per hectare (q/ha), number of seeds per fruit and germination percentage as per descriptors of the International Board for Plant Genetic Resources (IPBGR, 1990). The seed vigour index was calculated by using the formula given by Abdul and Anderson (1973). Ascorbic acid content (mg/100g) was assayed as described by Rangana (1976) and total phenol content (mg/100g) as per procedure given by Thimmaiah (1999). The D^2 statistic, as introduced by Mahalanobis (1936), was employed to evaluate genetic divergence among genotypes for both quantitative and qualitative traits. D^2 analysis is a valuable tool for determining the degree of genotypic divergence between biological populations and determining the relative contributions of different components to the total divergence, both within and between clusters. The grouping of genotypes was carried out utilizing Tocher's method as outlined by Rao (1952). Statistical analyses were done using INDOSTAT software.

Results

Analysis of variance for various characters revealed significant differences for all parameters under study. Mean performance of 30 genotypes for all traits are presented in Supplemental Table 1. After calculating the D^2 values for every potential pair, the 30 genotypes were categorized into eight groups based on their genetic configurations. This clustering highlighted considerable genetic diversity among the genotypes (Table 2) and (Figure 1). Cluster I constituted the largest group with eight genotypes, followed by Cluster II and IV, which each encompassed six genotypes. Cluster VI had four and Cluster VII had three genotypes. Clusters III, V and VIII comprised only one genotype each (mono-genotypic clusters).

Average inter- and intra-cluster distances in pink brinjal genotypes

Inter- and intra-cluster distances were calculated using the formulae described by Singh and Chaudhary (1977), serving as indicators of genetic diversity among clusters, as shown in Table 3. Inter-cluster distances surpassed intra-cluster distances, indicating a substantial level of

Table 1. List of genotypes of pink brinjal (*Solanum melongena* L.) used in this study and their sources.

No.	Genotype	Biological status	Source
1	SJPB-22-01	Advanced breeding line	SKUAST-Jammu, India
2	SJPB-22-02	Advanced breeding line	SKUAST-Jammu, India
3	SJPB-22-03	Advanced breeding line	SKUAST-Jammu, India
4	SJPB-22-04	Advanced breeding line	SKUAST-Jammu, India
5	SJPB-22-05	Advanced breeding line	SKUAST-Jammu, India
6	SJPB-22-06	Advanced breeding line	SKUAST-Jammu, India
7	SJPB-22-07	Advanced breeding line	SKUAST-Jammu, India
8	SJPB-22-08	Advanced breeding line	SKUAST-Jammu, India
9	SJPB-22-09	Advanced breeding line	SKUAST-Jammu, India
10	SJPB-22-10	Advanced breeding line	SKUAST-Jammu, India
11	SJPB-22-11	Advanced breeding line	SKUAST-Jammu, India
12	SJPB-22-12	Advanced breeding line	SKUAST-Jammu, India
13	SJPB-22-13	Advanced breeding line	SKUAST-Jammu, India
14	Jammu Sel-01	Local landrace	Gajansoo, Jammu, India
15	Jammu Sel-02	Local landrace	Marh, Jammu, India
16	Shalimar Hybrid-1	Public sector hybrid	SKUAST-Kashmir, India
17	Baramulla Local Sel-01	Local landrace	Parihaspura, Kashmir, India
18	Baramulla Local Sel-02	Local landrace	Pattan, Baramulla, India
19	Shalimar Local	Local landrace	Dargah, Kashmir. India
20	Ganderbal Local Sel	Local landrace	Ganderbal, Kashmir, India
21	Long Special	Private sector hybrid	Jyoti Agritec, Jammu, India
22	Kashmiri Long	Private sector hybrid	Jyoti Agritec, Jammu, India
23	Long Kashmiri	Private sector hybrid	Gulshan Seeds, Jammu, India
24	Lal Gulab	Private sector hybrid	Rajdhani Seeds, Jammu, India
25	Pink Long	Private sector hybrid	Shatabdi Seeds, Jammu, India
26	PPL-1823	Private sector hybrid	Sultan Seeds, Jammu, India
27	Manjhi	Private sector hybrid	Kalash Seeds, Jammu, India
28	Pink Raja	Private sector hybrid	Truegenic Seeds, Jammu, India
29	Nisha	Private sector hybrid	HM Clause, Jammu, India
30	Brinjal No. 704	Private sector hybrid	MAHYCO, Jammu, India

Table 2. Clustering of 30 genotypes of pink brinjal (*Solanum melongena* L.) based on D^2 statistics

Cluster	No. of genotypes	Genotypes
I	8	SJPB-22-01, SJPB-22-02, SJPB-22-04, SJPB-22-05, SJPB-22-07, Shalimar Local, Kashmiri Long
II	6	SJPB-22-03, SJPB-22-12, SJPB-22-13, Ganderbal Local Sel, Jammu Sel-01, Jammu Sel-02
III	1	SJPB-22-09
IV	6	Lal Gulab, Manjhi, Long Kashmiri, SJPB-22-08, SJPB- 22-11, Brinjal no. 704
V	1	Pink Raja
VI	4	Baramulla Local Sel-01, Pink Long, Shalimar Hybrid-01, Baramulla Local Sel-02
VII	3	SJPB-22-06, SJPB-22-10, Long Special
VIII	1	Nisha

genetic diversity among the studied genotypes. The highest intra-cluster distance was observed in Cluster VII (42.78), with Clusters VI and IV following closely at 40.27 and 39.57, respectively. Clusters III and IV exhibited the greatest inter-cluster distance (103.96), followed by Clusters III and VI (98.96), and Clusters III and VII (93.04). The proximity between Clusters I and III, as evidenced by the minimum inter-cluster distance of 40.17, suggests a stronger relationship among the genotypes within these clusters.

Cluster means for 18 characters in pink brinjal genotypes

Table 4 displays cluster means for each of the 18 characters studied. The single genotype in Cluster III had the maximum cluster mean value for germination percentage (87.33%) and seed vigour index (1115.67). The maximum cluster mean value for fruit length (15.81cm), unmarketable fruit yield per plant (0.32kg), fruit yield per hectare (303.8q/ha and number of seeds

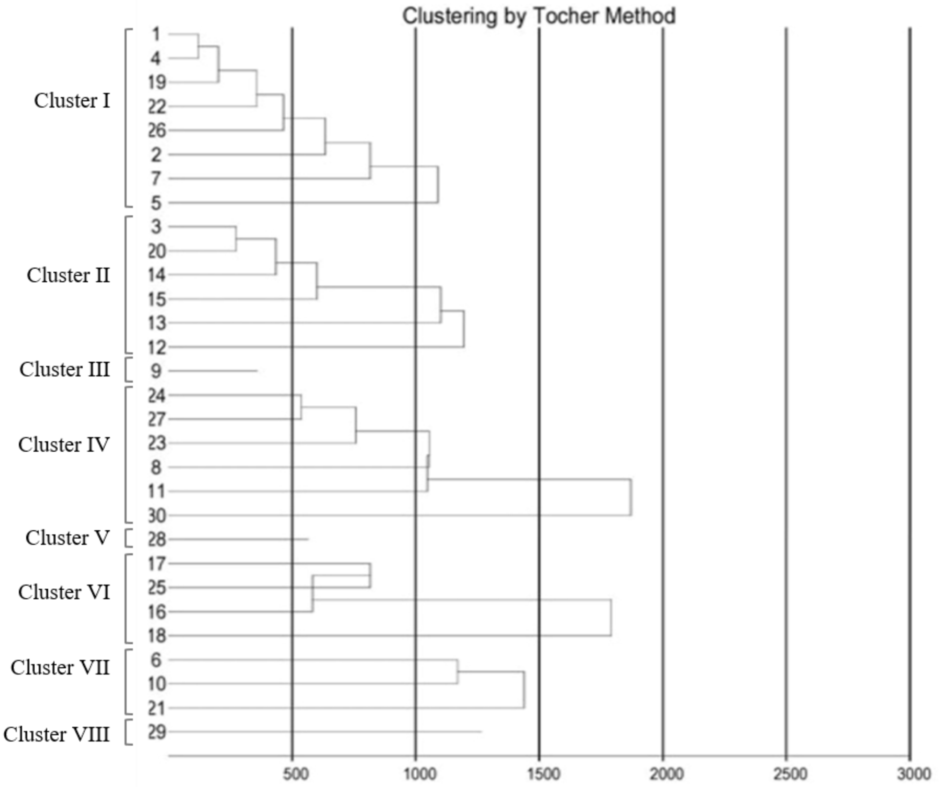

Figure 1. Dendrogram representing clustering pattern of 30 genotypes of pink brinjal (*Solanum melongena* L.). The numbers correspond to genotypes used in this study (see Table 1).

Table 3. Mean intra-cluster distance (highlighted in bold) alongside the inter-cluster distance values (D^2 values) for eight clusters among 30 genotypes of pink brinjal (*Solanum melongena* L.).

Cluster	I	II	III	IV	V	VI	VII	VIII
I	**28.47**	46.14	40.17	85.92	40.31	72.69	90.16	42.50
II		**33.33**	71.96	69.31	58.99	56.55	92.26	52.63
III			**0.00**	103.96	51.53	98.96	93.04	46.59
IV				**39.57**	80.81	60.29	62.55	64.29
V					**0.00**	61.75	78.35	45.61
VI						**40.27**	88.34	70.29
VII							**42.78**	64.53
VIII								**0.00**

per fruit (275.11) was observed in Cluster IV. The minimum cluster mean value for days to first flowering (37.00) and days to first harvest (62.33) was observed in Cluster V, whereas desirable cluster mean values for marketable fruit yield per plant (2.05kg) and total fruit yield per plant (2.36kg) were observed in Cluster VI. Cluster VII exhibited the highest mean values among clusters for fruit weight (125.22g), as well as for ascorbic acid content (12.41mg/100g) and total phenol content (2.22mg/100g). Cluster VIII had the maximum cluster mean value for plant height (70.00cm), number of branches per plant (7.33), plant spread (70.00cm^2), number of fruits per plant (31.00) and fruit diameter (5.23cm).

Relative contribution of various characters towards divergence

The percentage contribution to genetic divergence by all 18 characteristics is provided in Table 5. Observations revealed that the highest contribution to total divergence was from total fruit yield per plant (33.56%), followed by the number of seeds per fruit (21.38%), seed vigour index (7.94%), marketable fruit yield per plant (7.62%), fruit yield per hectare (3.61%), number of branches per plant (2.52%), days to first flowering (2.25%), plant spread (2.24%), unmarketable fruit yield per plant (2.21%), fruit diameter (2.07%), fruit length (1.89%), fruit weight (1.84%), total phenol content (1.61%), days to first harvest (1.13%),

Table 4. Cluster mean values for different characters in pink brinjal (*Solanum melongena* L.). The lowest values are highlighted in bold, and the highest values are in italic. DFF, days to first flowering; DFH, days to first harvest; PH, plant height (cm); NPB, number of branches per plant; PS, plant spread (cm^2); NFP, number of fruits per plant; FL, fruit length (cm); FD, fruit diameter (cm); FW, fruit weight (g); MFYP, marketable fruit yield per plant (kg); UMFYP, unmarketable fruit yield per plant (kg); TFYP, total fruit yield per plant (kg); FYH, Fruit yield per hectare (q/ha); NSF, number of seeds per fruit; G (%), germination (%); SV, seed vigour index; AA, ascorbic acid content (mg/100g); TPC, total phenol content (mg/100g).

Characters	I	II	III	IV	V	VI	VII	VIII
DFF	41.17	40.28	38.00	40.33	**37.00**	38.92	*41.67*	41.33
DFH	67.38	67.11	64.67	66.83	**62.33**	63.75	*70.44*	63.00
PH	63.83	67.56	61.00	63.78	65.33	**57.00**	59.56	*70.00*
NBP	5.71	**5.06**	6.67	6.28	7.00	6.50	6.11	*7.33*
PS	63.83	67.56	61.00	63.78	65.33	**57.00**	59.56	*70.00*
NFP	23.21	**19.89**	26.67	26.56	29.33	27.75	25.67	*31.00*
FL	11.58	13.94	**8.83**	*15.81*	9.00	14.50	11.22	10.00
FD	3.86	3.97	3.67	4.31	**3.63**	4.63	**3.63**	*5.23*
FW	63.04	77.50	**60.00**	117.22	69.00	86.00	*125.22*	70.00
MFYP	1.30	1.36	1.33	1.71	1.78	*2.05*	**1.26**	1.42
UMFYP	0.14	0.17	**0.07**	*0.32*	0.24	0.31	0.29	0.17
TFYP	1.44	1.53	**1.40**	2.05	2.02	*2.36*	1.56	1.60
FYH	212.91	226.04	**207.40**	*303.85*	299.74	249.56	230.61	236.53
NSF	224.79	256.44	**207.33**	*275.11*	209.33	260.50	222.56	250.00
G (%)	84.46	83.72	*87.33*	80.67	82.33	79.17	**78.11**	85.00
SV	1,030.33	998.39	*1,115.67*	891.61	919.67	848.25	**841.33**	1,050.00
AA	**10.06**	11.05	12.33	11.73	10.22	10.31	*12.41*	10.94
TPC	1.29	1.70	2.04	1.87	**1.15**	1.26	*2.22*	2.01

germination percentage (1.61%) and ascorbic acid content (0.69%).

Discussion

The highest intra-cluster distance was observed in Cluster VII while the greatest inter-cluster distance was observed between Clusters III and IV. The inter-cluster distance was larger than intra-cluster distance suggesting that genotypes within and between clusters were homogenous and diverse, respectively (Pawar *et al*, 2013). The data clearly showed that the clustering of genotypes was not influenced by their geographical distribution. Overall, the distribution pattern of genotypes from different regions into distinct clusters appeared to be random. Several studies (Mangi *et al*, 2020; Silambarasan *et al*, 2020; Kaur *et al*, 2021; Mohanty *et al*, 2021; Verma *et al*, 2021; Anbarasi and Haripriya, 2021; Chaitanya, 2022) have all documented similar findings in their respective research concerning brinjal. One potential explanation may be that it is very challenging to establish the precise place of origin of a genotype. The frequent and open exchange of genetic material among breeders within the country complicates the maintenance of a genotype's true identity. The lack of correlation between genetic diversity and geographical distance suggests that factors beyond geographical origins, like genetic exchange, drift, mutation, variation, and selection, could account for the observed genetic diversity. An alternative explanation could be that the diversity estimates derived from the characters examined in this study might not fully capture the variability influenced by additional physiological or biochemical characteristics, which could be significant in representing the overall genetic diversity within a population. Consequently, the selection of genotypes for hybridization should prioritize genetic diversity over geographical divergence.

Out of eight clusters, Cluster VIII had the highest mean value for plant height, number of branches per plant, number of fruits per plant and fruit diameter, implying that genotypes in this cluster can be selected directly on the basis of these features and employed in hybridization programmes. Cluster VI also showed the highest mean value for some important traits, namely marketable fruit yield per plant and total fruit yield per plant. The genotypes belonging to this cluster can be used in breeding programmes to develop high fruit-yielding hybrids. Cluster V had the lowest value for days to first flowering and days to first harvest, implying that the genotypes in this cluster are likely to be early maturing types. Hence, these genotypes can be directly employed for developing early crops. Cluster VII exhibited the highest mean value for ascorbic acid and total phenol content along with fruit weight. This implies that this cluster's genotype should be rich in nutritive value, and these genotypes should be employed in quality breeding. The genotypes in Cluster III had the highest cluster mean value for germination percentage and seed vigour index, indicating that genotypes from this cluster exhibit rapid and uniform crop establishment and growth across diverse environmental conditions.

Table 5. Percentage contribution of various traits towards genetic divergence in pink brinjal (*Solanum melongena* L.)

Rank	Characters	Percentage contribution (%)
1	Total fruit yield per plant (kg)	33.56
2	Number of seeds per fruit	21.38
3	Seed vigour index	7.94
4	Marketable fruit yield per plant (kg)	7.62
5	Fruit yield per hectare (q/ha)	3.61
6	Number of fruits per plant	3.34
7	Plant height (cm)	2.99
8	Number of branches per plant	2.52
9	Days to first flowering	2.25
10	Plant spread (cm^2)	2.24
11	Unmarketable fruit yield per plant (kg)	2.21
12	Fruit diameter (cm)	2.07
13	Fruit length (cm)	1.89
14	Fruit weight (g)	1.84
15	Total phenol content (mg/100g)	1.61
16	Days to first harvest	1.13
17	Germination (%)	1.05
18	Ascorbic acid content (mg/100mg)	0.69

A similar comparison of clusters based on the range of mean value of each character was done by earlier works, namely Ravali *et al* (2017); Banerjee *et al* (2018); Sindhuja *et al* (2019); Silambarasan *et al* (2020); Balasubramaniyam *et al* (2021); Kaur *et al* (2021); Verma *et al* (2021); Chaitanya (2022) .

Relative contribution of various characters towards divergence

The highest contribution to genetic divergence was observed from total fruit yield per plant. Many researchers including Ravali *et al* (2017); Bhushan *et al* (2018); Sindhuja *et al* (2019); Silambarasan *et al* (2020); Balasubramaniyam *et al* (2021); Mohanty *et al* (2021); Chaitanya (2022) confirmed the substantial contribution of fruit yield per plant to divergence.

The results indicate significant potential for developing new varieties of pink brinjal with increased yield and enhanced economically important attributes by utilizing this elite germplasm. In crop improvement initiatives, effective outcomes could be achieved through interbreeding among genotypes displaying exceptional mean performance for these traits.

Conclusion

Analysis of 18 traits using the Mahalanobis D^2 statistic demonstrated significant diversity among 30 pink brinjal genotypes, resulting in their classification into eight separate clusters. Mahalanobis D^2 proved to be an effective tool in clustering genotypes phenotypically and geographically. The genotypes within Clusters III and IV, which exhibit maximum inter-cluster distance, hold potential for utilization as parents in recombination breeding programmes as these are very diverse from each other. They could be employed to develop highly heterotic F_1 hybrids or to generate a broad range of transgressive segregants within populations, thereby facilitating the development of high-yielding varieties of pink brinjal. For recovering improved progenies for yield and quality characters, crosses can be attempted between the genotypes belonging to Clusters VI and VII as they show the highest cluster mean value for yield and quality parameters.

Supplemental data

Supplemental Table 1. Mean values of 18 different characters for 30 genotypes of pink brinjal (*Solanum melongena* L.) described in this study.

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Author contributions

Vishwash Bandhral played a crucial role in drafting the research programme and objectives along with Dr. Anil Bhushan, collecting different genotypes of pink brinjal from different sources, proper sowing and transplanting of field trial, recording field and lab parameters, analyzing data and concluding the results. Dr. Anil Bhushan assisted in drafting the problem of genetic divergence and formulating the objective, Dr. R.K. Samnotra reviewed the trail of divergence, Diksha Rani helped with data collection, and Sonali Sharma assisted with data analysis.

Conflict of interest

Authors have declared that no competing interests exist.

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Genetic diversity and intraspecific mitochondrial DNA variations in the Georgian Mountain breed of *Bos taurus* reveal admixture, introgression and potential parallel vs. convergent evolution patterns

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Abstract: This study elucidates the haplotype diversity and mechanisms of evolutionary divergence for a broad population of the Georgian Mountain breed (GMB) of *Bos taurus*, using the sequencing and analysis of its mitochondrial DNA (mtDNA). In the evolutionary analyses, sequences of the targeted mtDNA region, involving the D-loop, *CYTB*, tRNA-Thr, and tRNA-Pro encoding genetic loci were analyzed using MEGA11, DnaSP, and SplitsTree software packages. A total of 25 haplotypes were determined among 82 individuals of GMB, belonging predominantly to the haplogroups T (T3, T1, T2, T4) or Q (Q1). Ten singleton haplotypes could also be determined in the GMB population. In the maximum likelihood evolutionary analysis, the singleton haplotype SNGT-9 appeared to be most closely related to the *Bos indicus* sub-haplogroup I1a. The haplotype diversity (0.997), nucleotide diversity (0.00636) and the overall mean distance within a population (0.01) calculated for GMB were greater as compared to the respective estimates (0.930, 0.00482 and 0.00) determined for its closest cattle relatives globally, suggesting stronger selection. It is suggested that the GMB diversity has been shaped by both parallel and convergent evolution, as well as by possible introgression, while pinpointing this breed's ancient origin collectively.

Keywords: Georgian Mountain breed, cattle, haplotype, haplogroup, genetic diversity, population structure, mtDNA

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Introduction

Climate change, driven by global warming, threatens agricultural animals' survival in the Anthropocene (Elayadeth-Meethal *et al*, 2018). Smaller body size in mammals is thought to aid adaptation to warmer climates (Pacifici *et al*, 2017), as there appears to be an interplay between body size reduction and increased livestock tolerance to warming (Elayadeth-Meethal *et al*, 2018). In this light, preserving biodiversity, conserving endemic cattle breeds of small body size, and deciphering their population structures and evolutionary mech-

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anisms are important, given global food security pressures and climate change (Mitchell *et al*, 2018).

According to the archeozoological and genetic evidence, mainly relying on the results of mitochondrial DNA (mtDNA) analyses, modern cattle are thought to have emerged via just two independent and geographically distinct domestication events of aurochs (*Bos primigenius*), both of which occurred in southwest Asia: one event is thought to have occurred in the Fertile Crescent, resulting further into modern taurine breeds of *B. taurus*, while the other event took place in the Indus Valley, leading to the emergence of modern zebuine breeds of its subspecies *B. indicus* (Achilli *et al*, 2008). The diversity of modern cattle has been structured largely into three major groups represented by Eurasian taurine, African taurine and Asian indicine cattle; these include different types of crosses, all combinations and intricacies (Kim et al., 2020), with various breeds exhibiting predominantly the macro-haplogroup T of *B. taurus* (Achilli *et al*, 2008).

While the genetic diversity of many cattle breeds from the above groups has been well characterized, there remains a significant lack of information specifically concerning the Georgian Mountain breed (GMB) of *B. taurus* endemic to the Caucasus region. GMB individuals are very small in body size, with the live weight varying from 220 to 280kg for mature cows, and from 270 to 370kg for bulls (Kunelauri *et al*, 2019). The coat colours of GMB individuals are black, black-and-white or red-and-white. Compared with other breeds from this region, GMB not only demonstrates stronger endurance and enhanced sustainability towards the above conditions but is also less susceptible to impoverished food (Kunelauri *et al*, 2019). This breed has been well adapted to the harsh climate and other conditions of the Caucasus mountains, and to its grazing lands with slopes that sometimes reach an angle of 45 degrees (Kunelauri *et al*, 2019). Two initial studies by Kunelauri *et al* (2019, 2022) analyzing the sequences of mtDNA loci versus a mitogenome in a very limited number of cattle individuals, revealed *B. taurus* haplogroups T and Q (branch Q1) and suggested the presence of some unknown haplotypes as well in this breed.

Our study aimed to characterize the haplotypes across broad GMB populations from the Khevsureti and Adjara regions of Georgia, and to gain initial insights into their evolutionary mechanisms by analyzing mtDNA genetic loci.

Materials and methods

Animal sampling

A total of 82 GMB individuals were sampled across the Khevsureti and Adjara regions of Georgia, as these regions have a high concentration of this breed, in 2019–2022, selecting unrelated animals based on pedigree information to minimize kinship. Only one individual was selected and sampled per village across the above regions. Specifically, hair follicle samples from the selected Khevsurian (n = 36) and Adjarian (n = 46) cattle were obtained for mtDNA extraction, PCR amplification, and sequencing.

Primer design, PCR amplification and sequencing of targeted mtDNA

The Tissue and Hair Extraction Kit, coupled with the DNA IQ^{TM} kit (Promega, Inc., Madison, WI, USA), and the Quick-DNA Microprep Plus Kit (Zymo Research, Inc., Irvine, CA, USA), were used for the extraction of mtDNAs from the hair follicle samples. The PrimerQuest^{TM} Tool (Integrated DNA Technologies, USA) was used to design primers for PCR amplification and sequencing of the targeted mitochondrial genome region. The designed forward and reverse primers (5'-CCAACAAACTAGGAGGAGTA-3' and 5'-CGCGGCATGGTAATTAAG-3') allowed us to amplify the 810-bp mtDNA region encompassing the genes encoding for tRNA-Thr, tRNA-Pro, as well as the *CYTB* gene and the D-loop loci across the above mitochondrial region from the selected GMB individuals. PCR conditions were 94°C for 5min, followed by 35 amplification cycles, each consisting of sequential incubation at 94°C (30s), 51°C (30s), and 72°C (1min and 20s), with the final 72°C (5min) extension.

The DNA sequencing of the PCR-amplified products was performed in both directions, using the BigDye Terminator v3.1. cycle sequencing kit (Applied Biosystems, Inc., Foster City, CA or Thermo Fisher Scientific) or the BrilliantDye^{TM} Terminator (v3.1) Cycle Sequencing Kit (NimaGen, Nijmegen, the Netherlands). The post-cycle sequencing reaction contaminants were removed by applying the ZR DNA Sequencing Clean-Up Kit (Zymo Research, Inc., Irvine, CA, USA). The 3100xl Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA, USA) was used to separate the labelled DNA fragments by size; the Geneious Prime v. 7.0.9 (Biomatters, Inc., Boston, MA, USA) and Sequencher v. 5.4.6 (Gene Codes, Corp. Ann Arbor, MI, USA) were utilized to edit and assemble the consensus sequences. All low-quality sequences were trimmed from both forward and reverse DNA sequence reads. The representative mtDNA sequences, obtained from the above DNA sequencing experiments, were submitted to, and are available under the accession numbers OR412787-OR412811, the GenBank database of the National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/).

Evolutionary analyses

The MEGA11 (v. 11.0.13) and DnaSP (v. 6.12.03) software packages were used to determine the population structures and evolutionary features of GMB individuals and their genetically closest cattle from different breeds of *B. taurus* and *B. indicus*. For these analyses, the respective mtDNA regions of 77 genetically closest cattle (Supplemental Table 1) were selected in the NCBI nucleotide database, using megaBLAST with the default parameters (Expected threshold - 10, Word size - 28, Match/mismatch scores - 1,-2, Gap costs - Linear, Extension - 2). The evolutionary analysis included representatives of each haplotype from GMB and its genetically closest cattle individuals preselected based on DNA identity, query coverage and E values from the BLAST analysis. Specifically, for evolutionary analyses, we selected the 491-bp mtDNA region, encompassing the *CYTB* and D-loop loci, as well as the genes encoding for tRNA-Thr and tRNA-Pro, which were shared exclusively by all these organisms. In this subset, we performed a random selection of the breed representatives from their country of origin, when they exhibited the BLAST-generated multiple identical hits to the query DNA sequences in the NCBI GenBank database. MEGA11 was used to determine specifically the shape parameter for the discrete gamma distribution to model evolutionary rates, as well as nucleotide substitution patterns and rates across the targeted mtDNA genetic loci as recommended previously (Tamura and Nei, 1993). In the MEGA analyses, Maximum Likelihood (ML) estimates of the transition/transversion bias were also determined, by applying the Kimura 2-parameter model (Kimura, 1980). Maximum Composite Likelihood (MCL) estimates of the pattern of nucleotide substitution were also determined as described by Tamura *et al* (2004). The ML method and the Tamura-Nei model (Tamura and Nei, 1993) were used to infer the evolutionary relationships between the GMB individuals and their genetically closest cattle individuals.

The haplotypes and their diversity, as well as polymorphic (segregating) sites, nucleotide diversity (Pi), invariable (monomorphic) versus variable (polymorphic) sites, and singleton variable versus parsimony informative sites across the 491-bp mtDNA region were determined using DnaSP. The haplogroup was determined for each GMB individual according to the haplogroup classification described previously (Xia et al., 2021). The degree of linkage disequilibrium (LD) (including the ZZ-value) was assessed as recommended by Rozas *et al* (2001). As the standardized measure of LD, the ZZ-value was derived from the squared correlation coefficient (r^2) between pairs of polymorphic sites, and the variance of the r^2 values across multiple loci.

The two-tailed Fisher's (F) exact test and the chi-square (Chi-sq) test were applied to determine whether the associations between polymorphic sites were significant, with Bonferroni correction additionally employed for these tests as implemented in DnaSP.

In the genetic recombination analysis, a minimum number of putative recombination events (Rm), and the coordinates of recombination hotspots were determined when detected by DnaSP across the targeted mtDNA regions of the cattle individuals. For re-examining recombination inferences, we also applied the SplitsTree (version 4.14.4) and RDP4 (Beta 4.96) software packages. Particularly, we used the method of split decomposition method, implemented in SplitsTree, to detect parallel nucleotide substitutions with conflicting evolutionary signals in the targeted mtDNA region in the targeted cattle populations. Every subset of the mtDNA sequences that encompassed such parallel changes conjointly displayed across a SplitsTree-generated parallelogram(s) was subjected to the Phi (Pairwise Homoplasy Index) test (Bruen *et al*, 2006) for measuring homoplasy. In the RDP4 analyses, searching for genetic recombination events, the RDP, GENECONV, BootScan, MaxChi, Chimaera, SiScan, and 3Seq recombination detection algorithms were employed. We used the stringent approach: the predetermined Bonferroni-corrected P-values only in a range of ≤ 0.05 were considered statistically significant for the significant breakpoint clusters (99%) if detected in these analyses.

Results

DNA sequence polymorphism and phylogenetic analyses

Based on sequencing of a 810bp sequence of mtDNA a total of 25 haplotypes were determined among 82 GMB individuals, using DnaSP. The haplotypes, haplogroups, genetic sub-lineages/clusters, and geographic distribution of these cattle individuals are presented in Table 1.

As shown, a great majority of the GMB individuals belonged to the haplogroup T3 (42.6%) followed by Q1 (12.1%), T1 (3.6%), T2 (15.8%) and T4 (1.2%), as well as ten unique SNGTs not associated with any previously described haplotype of *B. taurus*.

In Figure 1, we display the ML tree constructed using the nucleotide analysis of the 491-bp mtDNA region, elucidating the population structures of the GMB haplotypes, their genetically closest cattle individuals of *B. taurus* worldwide, and their genetic relationships.

The ML analysis resulted primarily in the distribution of the GMB haplotypes, including several SNGTs, and their genetically closest cattle individuals across 13 major haplogroup-specific genetic clusters I-XIII (Figure 1 and Table 1). As shown, the mtDNA sequence profiles of specific GMB haplotypes represented by individuals 6K, 7K, 15K and 31A were identical to those of various breeds from the European, African and Southeast Asian regions, belonging to T3 haplogroup with $T3_{119}$ included. The phylogenetic inferences, for the GMB singletons, exhibited their broad scattering patterns across the ML tree, demonstrating their close genetic relationships with different breeds from different cattle groups globally. These groups included collectively the Turano-Mongolian, Central European, Podolic, European dairy, Balkan, British Mountain, South Asian and Shorthorned Zebu (*Bos indicus*) cattle respectively from haplogroups T1-T4, Q, and the specific sub-lineage I1a of the lineage I1. It is important to indicate that while many GMB haplotypes could not be distinguished globally from various *B. taurus* breeds within the T and Q sub-lineages, certain SNGTs of the above Georgian breed (e.g. SNGT-1, SNGT-4, SNGT-7, SNGT-9, SNGT-10) either clustered separately within or fell outside these sub-lineages (Figure 1).

Table 1. Sample designations and geographic regions for the Georgian Mountain breed (GMB) individuals, GenBank accession numbers for the sequenced mtDNA region of their representative haplotypes, as well as haplogroups and major genetic clusters. SNGT, Singleton; N/D, Not determined; GenBank accession # for a representative from each haplotype; QNSG, Q new sub-haplogroup.

No.	GMB ID	Region	GenBank ID	Haplotype	Haplogroup	Major Genetic Cluster
1	33KSh	Khevsureti	OR412798	T1-1	T1	VI
2	18K	Khevsureti	OR412803	T1-2	T1	IX
3	36KSh	Khevsureti	-	T1-2	T1	IX
4	13A	Adjara	OR412802	T2-1	T2	IX
5	12A	Adjara	-	T2-1	T2	IX
6	37A	Adjara	OR412800	T2-2	T2	VIII
7	23A	Adjara	OR412811	T2-3	T2	XIII
8	35KSh	Khevsureti	-	T2-3	T2	XIII
9	29KSh	Khevsureti	-	T2-3	T2	XIII
10	28KSh	Khevsureti	-	T2-3	T2	XIII
11	26KSh	Khevsureti	-	T2-3	T2	XIII
12	36A	Adjara	-	T2-3	T2	XIII
13	32A	Adjara	-	T2-3	T2	XIII
14	14A	Adjara	-	T2-3	T2	XIII
15	22K	Khevsureti	-	T2-3	T2	XIII
16	11K	Khevsureti	-	T2-3	T2	XIII
17	15K	Khevsureti	OR412808	T3-1	T3	XII
18	13K	Khevsureti	-	T3-1	T3	XII
19	39AB	Adjara	-	T3-1	T3	XII
20	1A	Adjara	-	T3-1	T3	XII
21	7K	Khevsureti	OR412806	T3-2	T3	XI
22	31KSh	Khevsureti	-	T3-2	T3	XI
23	6K	Khevsureti	OR412794	$T3_{119}$-1	$T3_{119}$	III
24	31A	Adjara	OR412795	$T3_{119}$-2	$T3_{119}$	IV
25	20A	Adjara	-	$T3_{119}$-2	$T3_{119}$	IV
26	10A	Adjara	-	$T3_{119}$-2	$T3_{119}$	IV
27	5A	Adjara	-	$T3_{119}$-2	$T3_{119}$	IV
28	14K	Khevsureti	-	$T3_{119}$-2	$T3_{119}$	IV
29	48AB	Adjara	-	$T3_{119}$-2	$T3_{119}$	IV
30	44AB	Adjara	-	$T3_{119}$-2	$T3_{119}$	IV
31	43AB	Adjara	-	$T3_{119}$-2	$T3_{119}$	IV
32	47AB	Adjara	-	$T3_{119}$-2	$T3_{119}$	IV
33	12K	Khevsureti	-	$T3_{119}$-2	$T3_{119}$	IV
34	2A	Adjara	-	$T3_{119}$-2	$T3_{119}$	IV
35	9A	Adjara	-	$T3_{119}$-2	$T3_{119}$	IV
36	18A	Adjara	-	$T3_{119}$-2	$T3_{119}$	IV
37	28A	Adjara	-	$T3_{119}$-2	$T3_{119}$	IV
38	40AB	Adjara	-	$T3_{119}$-2	$T3_{119}$	IV
39	46AB	Adjara	-	$T3_{119}$-2	$T3_{119}$	IV
40	10K	Khevsureti	-	$T3_{119}$-2	$T3_{119}$	IV
41	21K	Khevsureti	-	$T3_{119}$-2	$T3_{119}$	IV

Continued on next page

Table 1 continued

No.	GMB ID	Region	GenBank ID	Haplotype	Haplogroup	Major Genetic Cluster
42	8A	Adjara	-	$T3_{119}$-2	$T3_{119}$	IV
43	17A	Adjara	-	$T3_{119}$-2	$T3_{119}$	IV
44	27A	Adjara	-	$T3_{119}$-2	$T3_{119}$	IV
45	45AB	Adjara	-	$T3_{119}$-2	$T3_{119}$	IV
46	1K	Khevsureti	-	$T3_{119}$-2	$T3_{119}$	IV
47	17K	Khevsureti	-	$T3_{119}$-2	$T3_{119}$	IV
48	6A	Adjara	-	$T3_{119}$-2	$T3_{119}$	IV
49	16A	Adjara	-	$T3_{119}$-2	$T3_{119}$	IV
50	26A	Adjara	-	$T3_{119}$-2	$T3_{119}$	IV
51	34KSh	Khevsureti	-	$T3_{119}$-2	$T3_{119}$	IV
52	24A	Adjara	OR412796	T4-1	T4	V
53	2K	Khevsureti	OR412788	Q1-1	Q1	I
54	8K	Khevsureti	-	Q1-1	Q1	I
55	4K	Khevsureti	-	Q1-1	Q1	I
56	19K	Khevsureti	-	Q1-1	Q1	I
57	20K	Khevsureti	-	Q1-1	Q1	I
58	15A	Adjara	-	Q1-1	Q1	I
59	22A	Adjara	-	Q1-1	Q1	I
60	29A	Adjara	-	Q1-1	Q1	I
61	24KSh	Khevsureti	-	Q1-1	Q1	I
62	27KSh	Khevsureti	-	Q1-1	Q1	I
63	3A	Adjara	OR412789	QI-3	QNSG*	I
64	41AB	Adjara	-	QI-3	QNSG*	I
65	23Ksh	Khevsureti	-	QI-3	QNSG*	I
66	21A	Adjara	OR412791	II-1	N/D	II
67	19A	Adjara	-	II-1	N/D	II
68	38A	Adjara	-	II-2	N/D	II
69	30A	Adjara	OR412792	II-2	N/D	II
70	42AB	Adjara	OR412805	T3-3	T3	XI
71	4A	Adjara	-	T3-3	T3	XI
72	34A	Adjara	-	T3-3	T3	XI
73	3K	Khevsureti	OR412787	SNGT-1	Q	I
74	25A	Adjara	OR412801	SNGT-2	T	VIII
75	30KSh	Khevsureti	OR412810	SNGT-3	T2	XIII
76	35A	Adjara	OR412790	SNGT-4	N/D	N/D
77	33A	Adjara	OR412793	SNGT-5	N/D	N/D
78	25Ksh	Khevsureti	OR412797	SNGT-6	T1	VI
79	9K	Khevsureti	OR412799	SNGT-7	T3	VII
80	5K	Khevsureti	OR412807	SNGT-8	T3	XII
81	16K	Khevsureti	OR412804	SNGT-9	N/D	X
82	32KSh	Khevsureti	OR412809	SNGT-10	T2	XIII

Moreover, according to the ML analysis, certain genetic groups from the same sub-haplogroups of T (except T4), determined previously (Xia et al., 2021), fell into two or more distantly related phylogenetic clades.

In Table 2, we describe polymorphisms of the targeted mtDNA region of the GMB haplotypes exhibiting previously unknown mutations within the *B. taurus* global populations.

Specifically, a total of 11 unique DNA polymorphisms could be identified in this subset, being mainly G↔A and T↔C nucleotide substitutions. In addition, Table 3 displays the ML estimates of the substitution matrix, as well as the MCL estimates of the pattern of nucleotide substitution.

In Table 4, we provided the ML estimates of transition/transversion bias (R) and respective evolutionary distance values for the GMB haplotypes, their genetically closest cattle individuals, and these two cattle groups combined.

As shown, we only found some differences in the transition/transversion bias between the GMB haplotypes and their genetically closest cattle individuals worldwide: For GMB, the ML estimates (for A↔G and T [U] ↔ C) were collectively slightly higher in contrast to the MCL estimates (for T [U] ↔ C) being slightly lower as compared to these estimates determined for the group of its genetically closest cattle individuals worldwide. Similarly, some slight differences could be also found between the above cattle groups in the MEGA-generated values (Table 3) determined for I_d, the overall average composition and pairwise distances, the discrete gamma distribution, and the other parameters including average nucleotide composition, as well as nucleotide frequencies. However, as demonstrated, these values were still almost always greater for the GMB haplotypes as compared to their genetically closest cattle individuals. Furthermore, the average genetic distances within the population of the GMB and its genetically related cattle group were 0.01 and < 0.00, respectively. The calculated average distance between these two groups was 0.00557.

In the analysis of the targeted mtDNA region, using DnaSP, we could identify a 160-bp conserved region (the coordinates according to the *B. taurus* reference genome [NC_006853.1]: 15629-15788) exhibiting the genetic loci involved in coding for cytochrome b and tRNA-Thr. The other DnaSP-generated statistics are presented in Table 5.

As shown, in the DnaSP analysis, we could detect 12 polymorphic mutations across this mtDNA region collectively in the MGB haplotypes, which, in contrast, appeared to be monomorphic in the population of their genetically closest cattle individuals; and vice versa, the DnaSP identified four mutations that were polymorphic in the latter, while exhibiting the monomorphic patterns in the group of GMB haplotypes; a total of 12 mutations were shared by the above two groups; The average number of nucleotide differences (K) versus that of the

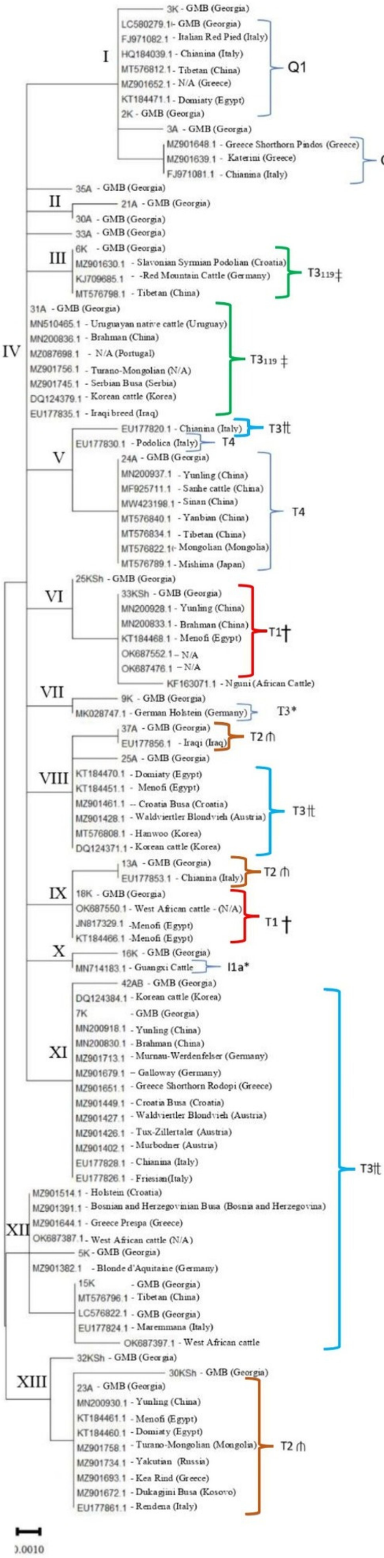

Figure 1. Maximum Likelihood (ML) tree showing the genetic relationships between randomly selected representatives of each haplotype of the Georgian Mountain breed and their genetically closest cattle individuals from the *Bos taurus* global populations identified by their corresponding GenBank accession IDs. I1a*, the sub-lineage of the I1 lineage of *Bos taurus indicus*. T3*, Haplogroup T3 exhibiting Holstein dairy cow described previously (Kinoshita *et al*, 2018).
T1†, T3, $T3_{119}$‡, T2, cow individuals sharing the same sub-haplogroups (T1,/T3/$T3^{119}$/T2) according to a previous study (Xia et al., 2021), although being clustered indifferent genetic groups across the ML tree.

Table 2. Previously unknown polymorphisms identified across the targeted mtDNA region of the Georgian Mountain breed haplotypes. The coordinates were determined according to the *B. taurus* reference mt genome (NC_006853.1) available in the NCBI eukaryotic genome database.

Haplotype	**Allele frequency**	**Polymorphisms and their coordinates across the targeted mtDNA region**										
		15789	15813	15846	15878	15917	15919	15954	15961	15966	16055	16057
T3-3	3	C	T	T	C	A	**G**	G	G	G	T	G
II-1	2	C	**C**	T	C	**G**	A	G	G	G	T	G
II-2	2	C	T	T	C	**G**	A	G	G	G	T	G
QI-1	13	C	T	T	C	A	A	G	**A**	G	T	G
SNGT-1	1	C	T	T	C	A	A	G	G	G	**C**	G
SNGT-2	1	**T**	T	T	C	A	A	G	G	G	T	G
SNGT-3	1	C	T	T	C	A	A	G	G	**A**	T	C
SNGT-4	2	C	T	**C**	C	A	A	G	G	G	T	G
SNGT-5	1	C	T	T	C	A	A	**A**	G	G	T	G
SNGT-8	1	C	T	T	**T**	A	A	G	G	G	T	A
SNGT-10	1	C	T	T	C	A	A	G	G	G	T	**T**

Table 3. Maximum Likelihood (ML) and Maximum Composite Likelihood (MCL) estimates calculated for the nucleotide substitutions of the targeted mtDNA region across the Georgian Mountain breed (GMB) haplotypes, their genetically closest cattle individuals globally, and both cattle groups combined. Rates of different transitional substitutions are shown in bold and those of transversal substitutions are shown in italics.

Targeted population		**ML estimate of substitution matrix**				**MCL estimate of the pattern of nucleotide substitution**			
		A	**T/U**	**C**	**G**	**A**	**T/U**	**C**	**G**
GMB	**A**	-	*1.13*	*0.98*	**10.5**	-	*2.07*	*1.79*	**10.14**
	T/U	*1.83*	-	**22.25**	*0.56*	*3.36*	-	**18.6**	*1.02*
	C	*1.83*	**25.66**	-	*0.56*	*3.36*	**21.45**	-	*1.02*
	G	**33.05**	*1.13*	*0.98*	-	**33.34**	*2.07*	*1.79*	-
GMB closest cattle relatives globally	**A**	-	*2.20*	*1.90*	**9.37**	-	*1.97*	*1.71*	**9.57**
	T/U	*3.57*	-	**19.59**	*1.08*	*3.21*	-	**20.03**	*0.97*
	C	*3.57*	**22.62**	-	*1.08*	*3.21*	**23.13**	-	*0.97*
	G	**30.90**	*2.20*	*1.90*	-	**31.56**	*1.97*	*1.71*	-
Both cattle groups combined	**A**	-	*1.25*	*1.08*	**10.54**	-	*2*	*1.73*	**9.74**
	T/U	*2.03*	-	**20.80**	*0.61*	*3.25*	-	**19.61**	*0.99*
	C	*2.03*	**24.01**	-	*0.61*	*3.25*	**22.64**	-	*0.99*
	G	**34.72**	*1.25*	*1.08*	-	**32.09**	*2*	*1.73*	-

nucleotide substitutions per site (Dxy) between GMB and the group of its genetically closest cattle individuals were 2.717 and 0.00553 respectively, while the net nucleotide substitutions (Da) per site between these two groups was -0.00006.

Analysis of evolutionary divergence mechanisms

To determine the mechanisms of evolutionary divergence of the GMB haplotypes and their genetically closest cattle individuals, the LD degree across the targeted mtDNA region was assessed. The scatter graphs (A-C), shown in Figure 2, provide the LD values plotted across the nucleotide distance estimates for both the above two groups separately and these groups combined.

Among the multiple LD-linked polymorphic sites in the mtDNA sequence alignment, different positions were identified across the cattle groups examined: for the GMB haplotypes (n = 26), the 12-338 site positions were highly supported by a strong F-generated p-value (< 0.001) and a Chi-square estimate of 26.000 ($p < 0.001$) after Bonferroni correction. For the cattle group genetically most closely related to GMB (n = 75), the sites 12-306, 12-338, 407-435 and 427-478 could be determined, strongly supported by the F-produced p-

Table 4. Results of the MEGA analyses of the targeted mtDNA region, elucidating the evolutionary patterns for the Georgian Mountain breed (GMB) haplotypes, their closest cattle individuals, and both cattle groups combined.

Evolutionary characteristics	**Targeted population**		
	GMB	**GMB genetically closest cattle globally**	**Both cattle groups combined**
Overall average disparity index (I_d)	0.0023750587	0.0012190602	0.0014805106
Overall average composition distance	0.0072881090	0.0048344067	0.0054175153
Average nucleotide composition for T(U)/C/A/G	25.1/21.8/40.8/12.4	25.1/21.7/40.8/12.4	25.1/21.7/40.8/12.4
Nucleotide frequencies (%) respectively for A/T(U)/C/G	40.76/25.09/21.76/12.39	40.78/25.11/21.74/12.37	25.1/21.7/40.8/12.4
ML estimate of transition/transversion bias (R)	6.23	4.01	7.69
Overall average pairwise distance	0.0064039174	0.0048494716	0.0052283806
Overall mean distance	0.01	0.00	0.01
Discrete gamma distribution	0.0500	0.1000	0.1000

Table 5. DnaSP-generated evolutionary statistics obtained from the nucleotide sequence analyses of the targeted mtDNA region for the Georgian Mountain breed (GMB) haplotypes, the group of their genetically closest cattle individuals, and both cattle groups combined. Singleton variable sites (2 variants)* - Site positions, in the DNA sequence alignment, for: GMB (174, 198, 231, 263, 304, 339, 346, 351, 389, 427, 440, 470, 472); Genetically most closely related cattle (309 345 389 441); Both cattle groups combined (174, 198, 231, 263, 304, 309, 339, 345, 346, 351, 440, 441, 472). Parsimony informative sites (2 variants)* - Site positions, in the DNA sequence alignment, for: GMB (12, 302, 338, 407, 434, 435, 443, 478,); Genetically most closely related cattle (12 306 338 407 427 434 435 443 470 478); Entire population (12, 302, 306, 338, 389, 407, 427, 434, 435, 443, 470, 478). Parsimony informative site (3 variants)* - Site position (442), in the DNA sequence alignment, for a group of GMB genetically closest cattle group. The DNA sequence alignment is provided in Supplemental Figure 1.

Evolutionary characteristics	**Targeted population**		
	GMB	**GMB closest cattle globally**	**Both cattle groups combined**
No. of polymorphic sites	22	15	26
Total No. of mutations	24	16	28
Average No. of nucleotide differences (K)	3.123	2.367	2.551
Nucleotide diversity (Pi)	0.00636	0.00482	0.00520
Theta (per site) from Eta	0.012	0.00667	0.008
Invariable (monomorphic) sites	469	476	465
Variable (polymorphic) sites	22	15	26
Singleton variable sites	13	4	13
Parsimony informative sites	9	11	13
Singleton variable sites (2 variants)*	13	4	13
Parsimony informative sites (2 variants)*	8	10	12
Singleton variable sites (3 variants)	0	0	0
Parsimony informative sites (3 variants)	0	1	0
Singleton variable sites (4 variants)	0	0	0
Parsimony informative sites (4 variants)	1	0	1
Sequence conservation (C)	0.947	0.947	0.947
No. of haplotypes (h)	25	20	34
Haplotype diversity (Hd)	0.997	0.930	0.950
Variance of haplotype diversity	0.00014	0.00013	0.00007
Standard deviation of haplotype diversity	0.012	0.011	0.008
DNA conserved region	13-173	13-173	13-173

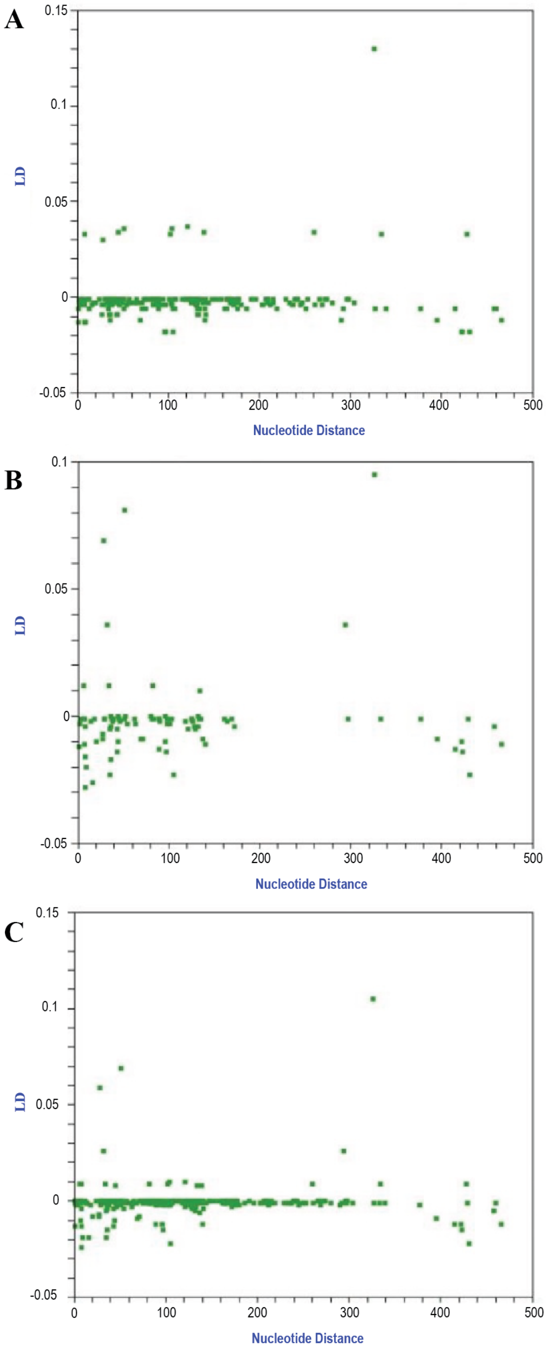

Figure 2. The LD patterns of the polymorphisms of the targeted mtDNA region for the Georgian Mountain breed (GMB) haplotypes (A), their genetically closest cattle individuals (B), and these two cattle groups combined (C).

values (< 0.001) and Chi-square estimates (26.172, 75.00, 42.391, 48.340; $p < 0.001$) after Bonferroni correction. For these two cattle groups combined ($n = 101$), the interlinked site positions 12-338, 407-435, and 427-478 could be determined and verified by the F-produced p-values (< 0.001) and the robust Chi-sq estimates (101.000, 43.148, 61.137; $p < 0.001$ respectively) after Bonferroni correction. The DnaSP-generated ZZ values, calculated for inferring intragenic recombination separately between the GMB haplotypes, their genetically most closely related conspecifics, and the total population, were -0.0236, -0.0044, and -0.0157 respectively. In the DnaSP analysis, $Rm = 1$, with the detected recombined regions located between the following sites for the above cattle groups respectively: 407-435, 427-478 and 389-434. In contrast, in the RDP4 analysis of the targeted mtDNA region, using RDP, GENECONV, BootScan, MaxChi, Chimaera, SiScan and 3Seq, we could not detect genetic recombination events that would be supported by statistically reliable values across the above entire population examined in this study.

When applying the method of split decomposition, in the SplitsTree analysis of the same mtDNA region, we could determine parallel nucleotide substitutions consolidated into five parallelograms shared by multiple individuals from the populations of the GMB haplotypes and their genetically closest cattle individuals. Importantly, as shown in Figure 3, while the highest fit value of 100 was obtained for the above split decomposition inferences, the bootstrap values, calculated for the nodes of these five parallelograms, were significantly lower, being ≤ 63.6. Moreover, the Phi test, when measuring homoplasy across the targeted mtDNA region for the above subset of cattle individuals, resulted in a very insignificant p-value of 0.4093.

Discussion

Haplotype diversity and population structures of GMB and its genetically closest cattle individuals

Most studies investigating the haplotype diversity and evolution of *B. taurus* have concentrated on the highly variable D-loop region of mtDNA (Colominas *et al*, 2015; Kunelauri *et al*, 2019). Although the D-loop is the most diverse functional region of the mitochondrial genome, several other genetic loci of the mitochondrial genome also show significant polymorphism. Nevertheless, when analyzing the DNA sequences of the D-loop region, some studies have struggled to consistently identify certain haplotypes (e.g. P and T5) in different *B. taurus* populations globally (Cubric-Curik et al., 2021). Furthermore, the analysis of the D-loop hypervariable loci has sometimes failed not only in distinguishing between specific breeds but also between some ancient branches (Achilli *et al*, 2009; Xia *et al*, 2019). DNA sequencing and analyses of the complete mitogenomes provided new and important insights into the genetic

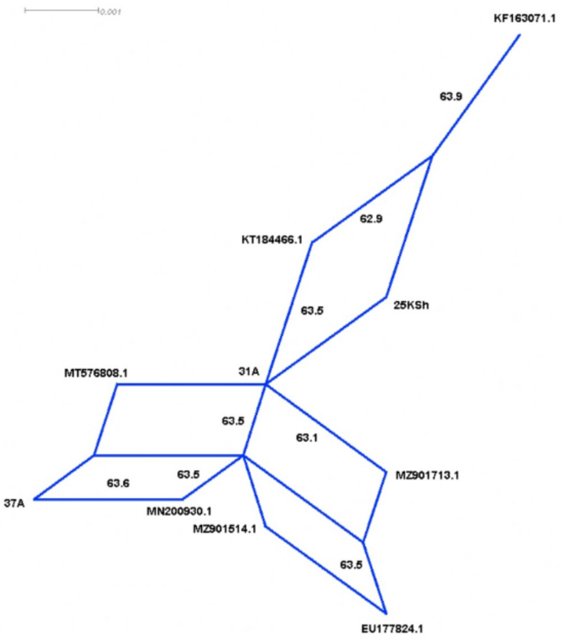

Figure 3. The SplitsTree-generated parallelograms showing the parallel nucleotide substitutions across the targeted mtDNA region, shared by some individuals of the Georgian Mountain breed (GMB) and their several genetically closest cattle individuals. The numerical values along the nodes of the parallelograms represent their bootstrap estimates obtained from 10,000 bootstrap replications. Fit = 100 for the above split decomposition inferences. In the splitsgraph, the GMB individuals are represented by the sample names assigned to these individuals (see Table 1), while their genetically closest relatives from the global cattle populations are displayed by their respective GenBank accession IDs.

diversity and evolution of *B. taurus* (Achilli *et al*, 2008; Xia *et al*, 2019; Xia et al., 2021; Cubric-Curik et al., 2021). However, the cost of DNA sequencing for complete mitogenomes is still not easily affordable for most low- and middle-income countries. In earlier studies, the DNA sequencing of *CYTB* loci also appeared very instrumental in revealing high haplotype variability (Tarekegn *et al*, 2018), genetic differences between specific breeds from different countries (Kim et al., 2013), and even male-mediated introgression (Kikkawa *et al*, 2003) in *B. taurus*. In our study, the DNA sequencing and analysis of the D-loop, *CYTB*, tRNA-Thr, and tRNA-Pro encoding genetic loci could not differentiate, in many instances, between GMB and multiple other breeds from the Turano-Mongolian, European taurine, and some other cattle within the T and Q sub-lineages. Although highlighting the necessity for examining complete mitogenomes, as discussed further, our findings also offer new insights into the haplotype diversity of GMB and the molecular-genetic mechanisms governing the evolution of this breed and its genetically closest cattle individuals.

Interestingly enough, according to the results obtained from our analysis of the targeted mtDNA region, the GMB haplotype diversity was notably greater than previously determined in two pilot studies. However, these pilot studies used a very limited sample size, with the initial investigation including 17 individuals (Kunelauri *et al*, 2019), and the subsequent one selecting 5 individuals from the original 17 (Kunelauri *et al*, 2022). Nevertheless, these authors (Kunelauri *et al*, 2022) reported on several GMB haplotypes falling outside of the known taurine diversity in their analysis of the complete mitogenomes of these cattle individuals. In our study, while the haplotype diversity estimate was comparatively smaller (0.997 versus 0.9995), the Pi value (0.00636) appeared to be notably greater than the mt genome-wide nucleotide diversity estimates (0.0015 and 0.0010-0.0020) calculated earlier respectively for taurine cattle from southeast Europe and some other European regions (Cubric-Curik et al., 2021). Thus, at least three different possible scenarios can be considered when attempting to explain the observed differences between GMB and the European cattle breeds: as compared with the latter, GMB may have an older evolutionary history, accumulating over time more genetic changes, and/or has evolved at higher evolutionary rates; It is also possible that, compared with GMB, the southeast European breeds might have undergone more extensive selection. These observations could pave a new avenue for future research to better understand the selection-driven evolutionary differences that can potentially exist between these two groups of cattle.

The domestication of *B. primigenius*, which took place around 10,000 years ago, marked a significant Neolithic advancement (Bonfiglio *et al*, 2010). This process involved cattle breeding over various periods and had profound socioeconomic impacts on Old World populations (Clutton-Brock, 1989), possibly including early tribes living in Georgia. It's worth noting that historically, dating back 1.8 million years, the territory of Georgia was inhabited by a variant(s) of *Homo erectus* (Lordkipanidze *et al*, 2013; Schwartz *et al*, 2014). In this light, it is possible that the above geographic region, being the habitat for certain early tribes, could be one of the oldest cattle domestication sites in the Old World. This scenario gains plausibility given the ancient traditions of winemaking (Ieri *et al*, 2021), honey production (Kvavadze *et al*, 2007) and wheat cultivation (Gogniashvili *et al*, 2021) in Georgia. Unfortunately, there is no clear scientific information available about ancient animal husbandry practices and traditions in Georgia.

Here, we show that the GMB Khevsurian and Adjarian populations belong predominantly to the T1, T2, T3 and T4 sub-haplogroups. It should be noted that the investigations, examining complete mitogenomes, revealed significant diversity across modern cattle within T (Cubric-Curik et al., 2021). The T sub-haplogroups primarily reflect specific geographical structuring, with T1 being most common in African breeds, T2 prevalent in Near Eastern and Mediterranean breeds, T3 predominantly found in European breeds, and T4 most frequently characteristic of East Asian breeds (Carvajal-Carmona *et al*, 2003; Chen et al., 2010). The earlier studies reported that, in Europe, T3 demonstrated a reduced diversity relative to Near Eastern cattle – consistent with the patterns found in European cattle in the Near East (Troy *et al*, 2001; Carvajal-Carmona *et al*, 2003). In our study, two unique haplotypes, representing the SNGTs, could be identified among the GMB individuals that belonged to T3. Interestingly, T4 could not be found previously in Near Eastern breeds within T (Troy *et al*, 2001). Here, we show that, similar to the East Asian and some other cow breeds, the GMB Adjarian population also can carry the sub-haplogroup T4 characteristics. According to our phylogenetic analysis, certain GMB SNGTs demonstrated their closest genetic affinities with some sub-linages within T and Q, while some others appeared to represent previously unidentified haplogroups of *B. taurus*. Importantly, the haplogroup Q, which is likely of Near Eastern origin, has been considered to be rare in the global populations of modern taurine cattle (Bonfiglio *et al*, 2010; Xia et al., 2021; Cubric-Curik et al., 2021). Here, we provide strong amplifying evidence for the growing existence of cattle individuals belonging specifically to Q1 – the sub-lineage of Q – across the GMB populations, strengthening the earlier findings of two pilot studies (Kunelauri *et al*, 2019, 2022). Moreover, our analysis of the targeted mtDNA region of the GMB populations revealed the possible presence of an unknown sub-haplogroup within Q. Thus, the present findings point out that Georgia should be added to the list of countries (such as Egypt, China, Turkey and several European countries), where the *B. taurus* populations exhibit Q along with other haplogroups (Achilli *et al*, 2009).

Among the typical representatives of the GMB Adjarian population, we could identify as well the mtDNA pattern that was most closely related to the sub-haplogroup I1a. I1a is a relatively novel sub-haplogroup of *B. indicus* (Chen et al., 2018), which originated in Indus Valley about 8,000 years ago, and further spread eastwards to Southeast Asia and southern China < 400 years ago (Loftus *et al*, 1994; Chen et al., 2010). Indicine cattle were found to be dominant in southern China (Li et al., 2013), which is considered to be one of the domestication centres. Interestingly, it is suggested that within I1 cattle found in Guangxi, I1a represents a unique and dominant sub-haplogroup while being absent in India, demonstrating the dominant status for local cattle in Yunnan located in the southwestern part of China (Xia *et al*, 2019). Thus, similar to earlier observations on cattle (Edwards et al., 2007; Achilli *et al*, 2008), the closest genetic affinity of the specific SNGT of GMB with I1a suggests the existence of local admixture populations in this breed, likely influenced by introgression from wild aurochs. Besides, the above highlights can be also collectively suggestive of the complex patterns of the domestication process contributing to shaping the GMB population structure; these findings are in strong agreement with the early studies on the complexity of the domestication process as being a phenomenon influencing the genetic diversity and divergence of cattle populations globally (Achilli *et al*, 2009; Bonfiglio *et al*, 2010; Olivieri et al., 2015; Xia *et al*, 2019).

Hence, considering all the above findings and observations, we suggest that GMB may have an ancient origin, playing an important role in the evolution of *B. taurus* globally. More extensive research, using at least the DNA sequencing of complete mitogenomes with significantly larger sample sizes, is needed to gain more in-depth insights into the evolution of GMB.

mtDNA Polymorphisms and mechanisms of evolutionary divergence of GMB

Examining the mtDNA polymorphisms of GMB, we could unravel the presence of multiple hitherto undescribed mutations, clearly exhibiting a transition bias, the phenomenon observed in Mesolithic wild aurochs (*B. primigenius*) (Edwards et al., 2007), as well as in some of the ancient and modern bovine populations (Stock *et al*, 2009). These mutations, identified across 11 sites of the targeted mtDNA region, appeared to be characteristic predominantly to certain GMB SNGTs, distinguishing them from other haplotypes determined previously in the *B. taurus* global populations. From the evolutionary patterns, it becomes clear that the observed heterogeneity is pronounced primarily across both the SNGT variable sites with two variants, and the parsimony informative sites with two and four variants, being coupled with the above evolutionary estimates including, but not limited to, h, Hd, $I_{d,}$ R and various overall average and mean distances values calculated.

This is the first study offering some important initial insights into the mechanisms of evolutionary divergence of the GMB populations. It should be noted that using the large-scale mitogenome sequence analysis of *B. taurus*, the first attempts aimed at detecting genetic recombination events in domestic taurine cattle revealed no evidence for this phenomenon in these animals (Cubric-Curik et al., 2021). While investigating the mechanisms of evolutionary divergence with split decomposition, we identified parallel nucleotide substitutions with some illuminating the conflicting evolutionary signals across the targeted mtDNA region in the populations of both GMB and its genetically closest cattle individuals identified worldwide. While usually such SplitsTree-derived signals can frequently exhibit lateral genetic transfer events, these putative homologous recombination inferences could not be strongly supported by the fit and bootstrap values in the subsequent analysis using the above software package. The scenario of homologous recombination was neither supported by the Phi test estimates when measuring homoplasy for the same mtDNA subset, suggesting, instead, most likely the presence of parallel or convergent evolution in these *B. taurus* populations. The lowered LD rates, calculated for the targeted mtDNA region of the above populations, could be additionally linked to possible recombination events across the populations of GMB and its genetically closest cattle individuals globally. However, it is important to consider that by lowering LD, homoplasy can sometimes mimic such recombination events (Tibayrenc and Ayala, 2017). Importantly, this and alternatively the other genetic recombination inferences (e.g. Chi-sq and Rm estimates) – the scenario of intragenic recombination – were also rejected when the negative ZZ values were considered in the DnaSP analyses. Such a similar conflicting scenario, depicting the Chi-sq and Rm positive inferences versus the negative ZZ estimates, was described previously, suggesting that at least Rm can be sometimes inflated by parallel mutations not necessarily associated with genetic recombination (Rozas *et al*, 2001). Interestingly enough, in the earlier study (Groves and Shields, 1997), the *CYTB* genes of the takin (*Budorcas taxicolor*) and muskox (*Ovibos moschatus*) from the family Bovidae were assumed to have been impacted by convergent evolution. Also importantly, the yak/bison mitochondrial transfer, in light of the parallel accumulation of unique mutations of mtDNA, was also suggested (Zeyland *et al*, 2012). Therefore, our findings conjointly with the above observations strongly lead to the scenario(s) of parallel and/or convergent evolution in the populations of GMB and its genetically closest cattle individuals. These scenarios, versus the scenario of genetic recombination, become even more plausible if we also consider the negative outcomes received from the RDP4 analyses revealing the absence of recombination breakpoints in the mtDNA region of the targeted *B. taurus* populations.

Conclusions

The majority of GMB individuals from the Khevsureti and Adjara regions of Georgia belong to the sub-haplogroups T1, T2, T3 (including $T3_{119}$), T4 and Q1. They also exhibit multiple novel haplotypes, largely represented by SNGTs. Some of these SNGTs may belong to currently unidentified sub-haplogroups or even to previously unknown haplogroups of *B. taurus*. Notably, the haplogroup Q is common in the GMB populations, unlike many other breeds and populations of *B. taurus* worldwide. Additionally, the Adjarian population of GMB includes the SNGT-9 that is genetically closest to the sub-haplogroup I1a of *B. indicus*. It is suggested that parallel and/or convergent evolution, along with introgression, have shaped the GMB population structure in these regions of Georgia. Further in-depth research, particularly through the DNA sequencing of complete mitochondrial genomes from a significantly larger number of GMB individuals, is needed to better understand the origin of this breed and its potential role in the evolution of *B. taurus* global populations.

Supplemental data

Supplemental Table 1. The genetically closest cattle individuals of the Georgian Mountain breed included in the evolutionary analyses.

Supplemental Figure 1. ClustalX-generated multiple DNA sequence alignment utilized in the evolutionary analyses of the Georgian Mountain Breed and its genetically closest cattle individuals.

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Author contributions

Givi Basiladze and Leila Tabatadze: Conceptualizing the research, and performing the sampling and selection of GMB-specific types of cattle individuals, also contributing to drafting the manuscript; Ekaterine Gabashvili and Mariam Osepashvili: Performing the DNA sequencing and sequence assembly procedures; Marine Murskhvaladze: Performing the nucleotide quality analysis, also contributing to the DNA sequence assembly procedures. Mamuka Kotetishvili: Contributing to the conceptualization of the research, performing evolutionary analyses, and writing the main manuscript text. All the authors read and approved the final manuscript.

Conflict of interest statement

The authors declare that they have no competing interests.

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Genetic Resources

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Use of plant genetic resources in Yemen and suggestions for potential improvement

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Abstract: The study aimed to determine the present state of the use of plant genetic resources in Yemen during the period 2007–2023 and to identify potential improvements, relying on historical descriptive information as well as on primary and secondary information sources, with a focus on the case of the National Genetic Resources Center. For genebank-conserved accessions, the study identified limited achievements, the most important being the characterization and evaluation of 1,100 accessions of different crops. Significant progress was also made with the initiation of hybridization research programmes for various crops. However, the study identified several gaps and obstacles hindering the use of plant genetic resources and concluded with recommended measures to address them with the ultimate goal of effectively and efficiently using plant genetic assets to improve food and agriculture security in the country. In this regard, projects such as the 'Rainfed Agriculture and Livestock' funded by the World Bank, are taking steps to promote on-farm conservation of landraces of cereals and food legumes, including the establishment of Seed Producers Associations in five governorates.

Keywords: Agriculture, Food, Genetic Resources, Use, Yemen

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Introduction

Over the past few decades, efforts have increased in collecting, documenting and conserving genetic resources in various countries with their different plant, animal, forestry, pastoral and aquatic components, including microorganisms and invertebrates. These resources are considered a national wealth and an indispensable economic resource for any country, not only because of their importance in food security but also because they are vital for sustainable development. The Convention of Biological Diversity (UNEP, 1992) and the International Treaty on Plant Genetic Resources for Food and Agriculture (FAO, 2004) are two binding global agreements calling the countries for concerted efforts to ensure the conservation of and access to biological resources, including plant genetic resources (PGR), to facilitate their sustainable use, along with fair and equitable sharing of benefits arising out of their utilization.

Many countries, including Yemen, have recognized the importance of genetic resources and have set out to establish centres, specialized units and genebanks since the 1970s, especially in the field of PGR. Over the past few decades, it has been possible to carry out many surveys and collect seeds and other types of samples of many diverse plant crops and species for conservation and herbaria. These accessions show a distinctive and rich plant genetic diversity in the country reflecting topographic, geographic and climatic diversity as well as the diversity of ecosystems and farming systems.

The flora surveys in Yemen identified a total of 2,838 plant species, including 2,602 that grow naturally, 129 cultivated plant species, 107 introduced crops, and 608 species identified as endemic (whose presence is limited to Yemen only) or semi-endemic species (whose

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presence is limited to the Arabian Peninsula only) (Al-Khulaidi, 2013).

Aljarmouzi *et al* (2023) mentioned that the National Genetic Resources Center (NGRC) in Yemen plays a pivotal role in collecting, conserving, documenting and characterizing PGR. Currently, the genebank conserves 6,849 seed accessions from 45 different crops. These crops encompass cereals such as sorghum, maize, millet, wheat and barley, totalling 4,701 accessions. Moreover, the genebank safeguards an extensive collection of legumes (1,215 accessions), including lentils, beans, cowpeas, peas and fenugreek. Additionally, it conserves a diverse selection of vegetables (561 accessions), such as onions, tomatoes, chilli peppers, cucumbers, zucchini, mallows, radishes, eggplant and okra. NGRC's efforts extend to the conservation of oil-producing crops (97 accessions) such as sesame and peanuts, as well as less commonly utilized crops like black seed (*Nigella sativa*), henna (*Lawsonia inermis*), coriander (*Coriandrum sativum*), caladium (*Caladium bicolor*), arugula (*Eruca sativa*) and mustard (*Brassica juncea* L.).

Furthermore, in various regions, field genebanks have been established by agricultural research stations to complement NGRC's endeavours, holding an extensive assortment of crops ranging from date palms, mangoes, citrus fruits, almonds, grapes, bananas, papayas, as well as forage and forest species. As Aljarmouzi *et al* (2023) reported, a total of 917 accessions are maintained in field genebanks. These field genebanks are strategically located across different regions, including the northern, central and southern highlands, the Tihama coastal plains region, the southern coast region (Al-Kud, Abyan), and the Eastern Plateau region (Marib and Seiyun). Collectively, NGRC and its associated field genebanks demonstrate a coordinated commitment to the comprehensive collection, conservation, documentation and characterization of Yemen's national PGR.

The process of collecting, conserving, documenting and characterizing PGR is not a goal by itself, but it aims to achieve sustainable use of those resources. In this regard, the use of genetic resources, especially the materials conserved in seed and field genebanks as well as *in vitro* storage, has remained one of the most important challenges facing the management of agricultural research and genetic resource institutions (Aljarmouzi *et al*, 2023). According to the Yemen national report in the Second Global Plan of Action for Plant Genetic Resources (GP2) of the UN's Food and Agriculture Organization (FAO, 2011), the issue of genetic resource utilization was considered one of the most prominent weaknesses in the implementation of programmes and plans for PGR in Yemen (FAO, 2019b).

The extent to which collected and conserved seeds and other accessions of different plant species and crops in genebanks are being utilized remains unclear. This issue needs to be addressed through studying, understanding and tracking any changes that may affect it, especially considering the current conditions and transformations that the country is witnessing. Identifying the challenges and obstacles is crucial to developing a clear vision for advancing the use of PGR – an objective this paper aimed to achieve.

Materials and methods

In this study, the authors relied on historical descriptive information regarding the use of PGR. This was done by collecting and reviewing national and international documents, reports and other surveys and studies related to PGR, in addition to relying on authors' previous works, personal knowledge and experiences, as team members directly involved in the conservation and use of genetic resources in Yemen (Aljarmouzi *et al*, 2023).

To prepare this paper, the authors used the methodological approach followed by the FAO Commission on Genetic Resources for Food and Agriculture (CGRFA), and the guiding indicators approved for assessing the implementation of the GP2 (FAO, 2011). About 50 of those indicators were used, mostly to evaluate the current state of PGR use in Yemen during the period 2007–2023, according to the areas and activities included in the GP2, as well as the medium-term plan of the Agricultural Research and Extension Authority (AREA) in Yemen, and the annual plans of NGRC, operating under AREA, Dhamar. Based on its general objective, this current study assessed the utilization state of PGR, focusing on the most relevant aspects of four main themes: 1) notable achievements, 2) major changes and trends, 3) gaps and challenges, and 4) developing proposals for future PGRFA activities.

Results and discussion

Most important achievements

Expanding characterization and evaluation activities and developing collecting of germplasm

There is no doubt that improving the characterization and evaluation of the conserved accessions allows and increases the efficiency of their use, considering that proper documentation is required, also to register landraces and to enable their protection by property rights. AREA, through NGRC and its affiliated regional research stations and specialized research centres located in the different agroclimatic zones, has implemented many research programmes and projects related to the characterization and evaluation of local varieties of cereals, vegetables and fruit tree crops. The main studies carried out are summarized in Table 1 and their results were published in the *Yemeni Journal of Research and Studies* (YJARS), issued biannually by AREA.

Table 1. Research and studies published in the *Yemeni Journal of Research and Studies* (YJARS) on the characterization and evaluation of germplasm accessions in the Agricultural Research and Extension Authority (AREA) and its affiliated research stations and centres. Source: Prepared by the authors based on the articles published in the YJARS.

Title of the study	**Crop(s)**	**No. of accessions**	Reference
Evaluation of some local germplasm of lentil, collected from some areas located in the northern and central highlands	Lentil	22	(Lutf, 2000)
Comparing sorghum varieties for resistance to sorghum midge in the Tehama region of Yemen	Sorghum	5	(Muharram *et al*, 2001)
Shibam -2: a new faba bean variety with small-seed for the northern highlands region of Yemen	Faba Bean	1	(Lutf, 2000)
Genetic analysis of some traits of maize plants under water stress conditions	Maize	15	(Muqbil and Abdullah, 2002)
Evaluation of several local peanut germplasm accessions	Peanuts	9	(Sodi and Fouad, 2003)
Evaluation of grain yield of barley varieties with the participation of farmers in the northern highlands	Barley	8	(Saif, 2004)
Evaluating the productivity efficiency of some introduced maize hybrids	Maize	11	(Noman, 2004)
Evaluating of local landraces of eggplant in Wadi Hadhramaut	Eggplant	32	(Hassan, 2005a)
Evaluation of local landraces of carrots in Wadi Hadhramaut	Carrot	15	(Hassan, 2005b)
Grain yield comparison of wheat varieties in the Yemeni northern highlands with farmers participation.	Wheat	20	(Saif and Al-Shamiri, 2005)
Evaluation of yield and agricultural characters of eight wheat varieties	Wheat	8	(Saif *et al*, 2005)
Evaluation of local landraces of sweet melon in Wadi Hadhramaut	Watermelon	44	(Hassan, 2006)
Study of heritability percentage of some quantitative characters in F2 lentil hybrids	Lentil	12	(Zaid and Maqbool, 2006)
Yield efficiency of the wheat promising variety Qaa Elhaqal-7 in Yemeni central highlands region	Wheat	4	(Habib *et al*, 2007)
Evaluation of the local landraces of tomato in Wadi Hadhramaut	Tomato	25	(Hassan and bin Zeghew, 2007)
Purification and evaluation of the local wheat variety (Arabi)	Wheat	1	(Habib *et al*, 2007)
Evaluation of different varieties of fenugreek under rainy conditions in the northern highlands of Yemen	Fenugreek	5	(Zaid, 2007)
Evaluation of local genotypes of local pumpkins in Wadi Hadhramaut	Pumpkin	21	(Hassan and bin Zeghew, 2007)
Evaluation and description of local landraces of common bean	Beans	37	(Zaid *et al*, 2008)
Evaluation of genetic landraces of okra in Wadi Hadhramaut	Okra	31	(Hassan and bin Zeghew, 2008)
Evaluation of yield and other agronomical characters of local wheat mutants under rain-fed conditions	Wheat	9	(Saif *et al*, 2008)
Evaluation of the performance of five cowpea varieties under rain-fed conditions in the southern uplands of Yemen	Cowpea	5	(Al-Duwailah, 2009)

Continued on next page

Table 1 continued

Title of the study	**Crop(s)**	**No. of accessions**	Reference
Evaluation of radiated seeds of Sonalika wheat variety resistant to yellow rust	Wheat	1	(Basha *et al*, 2009)
Improving the quantitative and qualitative traits of Gemmiza-9 wheat variety by mutation induction	Wheat	3	(Saif *et al*, 2010)
Use of gamma rays to induce desirable mutants in local lentil variety (D2001)	Lentil	7	(Zaid and Saif, 2010)
Evaluation of the genotypes of coriander, nigella and cumin in Wadi Hadhramaut	Coriander, nigella and cumin	66	(Hassan and Al-Saqqaf, 2011)
Yield and quality evaluation of seven potato varieties in Wadi Hadhramaut	Potato	7	(Hassan *et al*, 2011)
Evaluation of different barley varieties for yield and other agronomical characters under rain-fed conditions	Barley	46	(Saif and Al-Shamiri, 2011)
Evaluation and selection of some introduced forage lines of common vetch for Yemeni northern highlands	Vetch	15	(Zaid, 2011)
Evaluation of yield and some agronomical traits of cowpea varieties under spate irrigation at Yemen southern coastal region	Cowpeas	15	(Al-Bakri and Sodi, 2012)
Early performance of some Mexican peaches and nectarines in Yemen highlands	Peach	12	(Al-Dalas *et al*, 2012)
Productivity evaluation of four finger millet cultivars (Eleusine coracana) under the central highlands conditions	Millet	4	(Doss *et al*, 2013)
Effect of mutants on quantitative and qualitative characteristics of local Bakkur barley under rain-fed conditions	Barley	10	(Saif *et al*, 2014)
Effect of pure line selection on the growth and yield characterization of local sorghum variety Qaira'a	Sorghum	1	(Noman and Dirham, 2014)
Morphological characterization of some plant genotypes of date palm in Shabwa Governorate, Yemen	Date palm	9	(Abdullah and Saeed, 2014)

Regarding the field collections, teams of researchers undertook the characterization of varieties of mango, coffee, date palm and grapes in different regions of Yemen. As a reference standard, the International Plant Genetic Resources Institute (IPGRI) descriptors were used. For example, in 2021, a team of researchers at the Northern Highlands Research Station (NHRS) characterized 45 genotypes (14 grape, 20 apricot, 3 pear, 4 fig and 4 peach genotypes).

In 2022, five grape varieties and five pomegranate varieties were characterized. The results of the cluster analysis confirmed the distinct diversity among grape and pomegranate varieties both of which are among the most popular fruit crops in Yemen (Al-Dalas *et al*, 2022).

The same is true for coffee, as several genotypes have been described in Sana'a Governorate. A team of researchers at the Al-Wadi and Al-Sahra Research Station (WSRS) in Seiyun, Hadramaut, also characterized date palm varieties in the Yemeni governorates where the crop is cultivated (AREA, 2013).

As for mango, all varieties grown in Yemen have been characterized, including 26 varieties introduced by the Ministry of Agriculture and Irrigation and AREA (Al-Munaifi, 2022). In this context, it is worth noting that documentation and description of mango varieties spread in Yemen were also implemented by AREA with the support of the Sultanic Dewan of Oman and included in the collection (AREA, 2013).

Upon reviewing the NGRC technical reports and the project reports implemented through the centre, specifically those focused on characterizing accessions stored in the genebank (Table 2), significant weaknesses were identified. These included inconsistency in the data and repetition in the description of some accessions.

For example, 1,967 sorghum accessions, more than 1,320 millet accessions, 883 wheat seed accessions and 918 barley seed accessions were characterized (Table 2), and the most prominent results of this characterization can be summarized as follows:

- Every report from NGRC included activities related to the phenotypic characterization of genetic resource accessions.
- There was a repetition in the characterization for some accessions because of the weak management operations in the genebank, especially in documentation. The number of wheat and barley accessions characterized exceeds the actual number of accessions preserved in the genebank.
- The inadequate quality and accuracy of characterization data were clearly demonstrated, as it was noted that these reports depend on a limited number of descriptors that were studied or documented.
- Some technical reports were prepared in a systematic manner and were scientifically sound, but they were not published in the form of evidence or scientific articles in peer-reviewed scientific journals.
- The results on varietal characterization were not documented with photos highlighting the characteristics of the local landraces and genotypes that were studied or referred to verbally in the reports.

In this context, it is also worth noting that some accessions of millet, wheat and sorghum were genetically characterized using molecular markers to determine their degree of genetic relatedness, through the 'Rainfed Agriculture and Livestock' project (2006–2013) and the 'Agricultural Biodiversity and Adaptation to Climate Change' project (2014–2015), both funded by the World Bank in collaboration with the International Center for Agricultural Research in the Dry Areas (ICARDA) and NGRC.

Encouraging crop diversity

The information collected for this paper, through reviewing the main and secondary references, revealed that the optimal use of genebank holdings can take different forms to encourage crop diversity utilization in the country. Some important examples of PGR use are summarized below:

- Agricultural farming systems that rely on a limited number of crops lack resilience, and these systems can lose their production because of factors such as diseases and pests (Lin, 2011). Therefore, diversification in crop production must be encouraged to meet local and national needs as well as to improve prevailing dietary patterns.
- In cooperation between FAO and AREA, quinoa (*Chenopodium quinoa*) was introduced and evaluated in farmers' fields, starting in 2013 with the introduction of several types of quinoa. Many genotypes were evaluated on the research farms in Sana'a, Taiz, Dhamar and Al-Kadan under different environmental conditions. In 2014 and 2015, the productivity of three varieties was evaluated at the research farm in the central highlands, to obtain a variety with high fodder and grain productivity suitable for the conditions of the region (Daws and Al-Muallem, 2018).
- The WSRS in Seiyun, Hadhramaut Governorate, has evaluated and propagated Haidawan (*Boerhavia elegans*), a naturally growing local plant with significant nutritional value. This plant is commonly used as a food ingredient in the dietary meals of the Hadhramaut community, as well as in the Al-Mahra and Shabwa Governorates. One notable dish that features Haidawan is the 'Aseed' meal, a porridge made with mashed dates and sesame oil, locally known as 'Salit Juljul'. Haidawan is rich in bioactive compounds, including antioxidants and essential nutrients, which contribute to its health benefits. In addition to its use in Aseed, Haidawan seeds are often ground into flour and incorporated into various traditional recipes. The seeds are also recognized for their potential to enhance the nutritional profile of foods such as biscuits (Kanzal and Madhi, 2012).
- Introducing varieties of beans, lentils, peas, wheat, and barley into governorates where the cultivation of such crops has declined because of economic and social factors, for example in Dhamar Governorate (in the districts of Jahran, Ans, Utomah, and Al-Hada), Sana'a Governorate (in the Bani Matar district), and Al-Mahwit

Table 2. Number of accessionsof the main crops in Yemen, preserved in the genebank at the National Genetic Resources Center (NGRC), Dhamar, and characterized and evaluated between 2007 and 2021. Source: Prepared by the authors based on the technical reports of NGRC (2007–2021).

Crop	**Year**										**Total**
	2007	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2019**	**2021**	
Sorghum	-	-	638	-	-	277	110	227	57	658	**1,967**
Millet	197	289	442	-	-	130	-	130	15	117	**1,320**
Maize	73	-	120	-	-	50	-	50	4	36	**333**
Wheat	61	-	202	154	-	97	-	97	35	237	**883**
Barley	57	-	332	84	-	112	-	110	7	216	**918**
Cowpea	59	-	170	-	-	88	-	18	18	-	**353**
Lentils	53	-	108	-	-	58	-	17	3	78	**317**
Peas	23	-	26	-	-	-	-	16	3	89	**157**
Beans	-	-	-	-	-	-	-	-	-	93	**93**
Total	**523**	**289**	**2,038**	**238**	-	**812**	**110**	**665**	**142**	**1,524**	**6,341**

Governorate (in Shibam and Al-Dhula'a districts), Ibb Governorate (in Yarim district), Hajjah Governorate (in Kuhlan Af'far District) and Amran Governorate (in Qa'a Al-Bawn). Expanding the cultivation of legume crops is one of the priorities of agricultural policymakers at present, especially considering war and siege conditions. It is worth noting in this context that the introduction and encouragement of the cultivation of these crops and their varieties have taken place through several projects, such as the 'Food Security Project', funded by the Islamic Development Bank (IsDB), the Kuwait Fund for Arab Economic Development and the State of Qatar. Additionally, the 'Participatory conservation and sustainable use of local landraces to improve the livelihood and resilience of farmers to climate change in Yemen' project, funded by FAO (FAO, 2019a), has introduced and spread legume varieties in areas with very limited legume cultivation. One of the most important obstacles facing the expansion of legume cultivation is the reluctance of farmers to grow these crops that need guarding and protection because people uproot the plants and eat the green immature pods or fruits directly, especially since the number of farmers who grow these crops is limited so far.

- Introducing varieties of almond and coffee and encouraging farmers in Sana'a Governorate (Haraz, Al-Haymah and Bani Matar districts) to grow these crop varieties to increase crop diversity (Y30, 2023). The same is also noted in the case of Ibb Governorate (Al-Saddah, Al-Nadira and Wadi Banna districts) and in Taiz Governorate (Al-Mawaset, Mawiya and Al-Shamaytain districts) (UNDP, 2021).
- In Dhamar Governorate (Utomah District), the Agriculture Office (AO) implemented many activities encouraging crop diversification. In addition to the expansion and spread of coffee and almond trees in the district stated above, new types of fruits have also been introduced, such as kiwi, blackberry, pomegranate, Sa'adi grape and Annona fruit crops. It was also noted that some innovative farmers in other governorates began individually to grow cardamom (Sana'a Governorate) and ginger (Al-Mahwit Governorate).
- The 'Rainfed Agriculture and Livestock' project funded by the World Bank in 2006–2013 included an activity related to promoting on-farm conservation and sustainable use of landraces of cereals, food legumes and vegetables, implemented jointly by ICARDA and AREA. This project provided examples of added-value technologies and alternative sources of income along with institutional arrangements. It has established 70 seed producer associations in five governorates to multiply and distribute seeds of landraces of sorghum, millet, wheat, barley, maize, lentil and faba bean.

Supporting breeding and genetic improvement programmes

The genetic material that has been – or is being – collected can be used to identify or select distinctive traits to develop crop varieties as needed, or to expand the genetic base of a breeding programme. Such traits may include earliness of the local varieties (of cereal and leguminous crops), or drought resistance and salt tolerance in some other local varieties, and good-quality attributes needed by breeding programmes to develop new high-yielding and climate-resilient varieties.

In this regard, the NGRC in Dhamar provided seed samples of many local varieties in response to requests from researchers and graduate students in various Yemeni agricultural regions to implement genetic improvement activities. However, these requests are still very limited compared to the available genetic material preserved in the genebank (Table 3).

Plant breeders at AREA agricultural research stations have implemented many breeding programmes using mutations through external projects funded by the International Atomic Energy Agency (IAEA), or through local projects funded by the annual government budget, and the work on these programmes is still ongoing. Distinctive successes have been achieved in this aspect, especially in sesame and barley. Three varieties of sesame were released: Hazza-1 and Hazza-2, both distinguished by their red seed colour and superior

Table 3. Crops and number of genebank accessions provided by NGRC upon request for research and breeding purposes. Source: Prepared by authors based on the data of the genebank of the National Genetic Resources Center in Dhamar.

Year	Crop	No. of accessions
2010	Lentil	5
	Sorghum	5
	Wheat	2
	Barley	4
	Lentil	5
	Bean	2
2011	Maize	10
2013	Millet	20
	Sorghum	175
	Millet	40
	Maize	37
	Wheat	34
	Barley	33
	Cowpea	35
	Lentil	19
	Fenugreek	14
	Peas	11
	Bean	10
	Maize	5
2014	Okra	35
	Wheat	24
	Cowpea	45
	Lentil	15
	Sorghum	2
	Beans	2
	Peas	1
	Total	**590**

productivity compared to local varieties, and Hazza-3, noted for its white seed colour, high yield and high oil content. Varieties of wheat, barley, fenugreek and lentils were also released in the northern highlands as part of the mutation programme.

Between 2013 and 2020, a total of 93 research activities were conducted in the field of variety evaluation and improvement across AREA branches, including regional research stations and national centres (Table 4).

In 2021, thanks to AREA, many varieties of several crops, including wheat, barley, corn, lentils, beans and peas were released by the High Committee for Registration of Agricultural Varieties and Technologies (HCRAVT), which includes representatives of various relevant authorities. These varieties were delivered by AREA to the public Corporation for Improved Seed Multiplication (GSMC) in a special ceremony sponsored by Ministry of Agriculture and Irrigation (Table 5).

The Genetic Resource Center (GRC) of the College of Agriculture, Sana'a University also implemented many activities such as the evaluation of landraces and breeding by selection method during the period 2014–2019, the most important of which were the following (personal communication, Dr Mohammed Al-Aswadi, GRC Director, College of Agriculture, Sana'a University, 2022):

- Production of maize hybrids
- Improvement of wheat productivity (Al-Himyari variety and Al-Bawni variety)
- Improvement of barley varieties productivity (Sakla and Al-Aswad (the Black))
- Improvement of four sorghum varieties productivity (Al-Lahmani, Al-Jameli, Al-Shahedhi and Al-Safara)
- Improvement of local lentils products
- Improvement of local peas productivity.

These advancements are integral to genetic improvement programmes aimed at enhancing traits such as earliness, drought resistance and heat tolerance. The productivity of these varieties has been significantly improved through the purification and maintenance of local varieties well-adapted to Yemen's predominantly arid climate. The improvement process involves selecting plant heads based on distinctive characteristics such as size, weight and yield.

Supporting seed production and distribution

To ensure the use of available PGR, effective seed systems must guarantee that the seeds adopted by farmers for cultivation are available in sufficient quantity, of high quality, and can be obtained by farmers at the right time, in the right place and at reasonable prices.

During the past decade, AREA and GSMC received funding from FAO to produce and distribute seeds to farmers as part of humanitarian aid. These seeds include locally adapted varieties such as sorghum, millet, barley and lentils, which are crucial for maintaining agricultural biodiversity and resilience. These local varieties, inherited by farmers from their ancestors, are well-adapted to climate change and represent an important genetic resource for improving agricultural production. Therefore, the multiplication and distribution of these local seeds were vital for preserving genetic resources and ensuring their sustainable use.

It should be noted here that after problems occurred due to distribution of low-quality seeds of cereal crops by some humanitarian aid organizations, the Ministry of Agriculture and Irrigation stipulated that organizations and providers of seed distribution services to farmers must have the seeds screened and packaged in the stores and warehouses of the GSMC. FAO's support and funding for AREA and GSMC came within the framework of strengthening its capabilities to fulfil any resulting obligations or requirements from the execution of the Ministry's new instructions.

However, there was an increase in cultivated areas and the production of improved seeds for certain crops during specific years (2014–2017). These seeds were distributed to farmers based on their needs and the availability of funding from supporting organizations. It is worth noting that despite fluctuations observed

Table 4. Research activities related tothe evaluation of crop varieties and genetic enhancement implemented by the Agricultural Research and Extension Authority (AREA) research stations and centres (2013–2020). Source: Prepared by authors based on the reports of a collection of multi-year annual technical reports of the different AREA research stations and centres (2013–2020). CHRS, Central Highlands Research Station; SHRS, Southern Highlands Research Station; NHRS, Northern Highlands Research Station; WCRS, West Coast Research Station; SCRS, Southern Coast Research Station; ECRS, Eastern Coast Research Station; WSRS, Wadi and Al-Sahra Research Station; ERRS, Eastern Region Research Station; NGRC, National Genetic Resources Center.

Research activity	**Research Station/Centre**									**Total**
	CHRS	**SHRS**	**NHRS**	**WCRS**	**SCRS**	**ECRS**	**WSRS**	**ERRS**	**NGRC**	
Evaluation of cereal crop varieties (wheat, barley, sorghum, maize and millet)	21	2	6	2	-	-	2	5	6	**44**
Evaluation of varieties of legume crops (lentils, beans, peas, chickpeas, beans and fenugreek)	8	2	5	-	-	-	-	-	4	**19**
Evaluation of varieties of vegetable crops (potatoes, onions and carrots)	2	-	-	-	-	-	3	-	-	**5**
Evaluation of varieties of fruit crops (almond, peach, mango, palm, lemon, banana and papaya)	2	-	1	2	4	-	1	-	-	**10**
Evaluation of cash oil crop varieties (peanuts, sesame, quinoa, cotton, safflower and fescue)	2	-	-	3	3	2	1	4	-	**15**
Total	**35**	**4**	**12**	**7**	**7**	**2**	**7**	**9**	**10**	**93**

Table 5. Species and varieties registered in the High Committee for Registration of Agricultural Varieties and Technologies (HCRAVT), Plant Production Directorate, Ministry of Agriculture and Irrigation (MAI), Sana'a. Source: Prepared by authors based on the minutes of HCRAVT meetings.

Crop	**No. of varieties**	**Names of varieties**
Wheat	10	Bohouth-8, Bohouth-14, Bohouth-15, Bohouth-37, Bohouth-5, Sonalika Mohasan, Bohouth-10, Arabi, Nagi, Shibam-8 and Naeem-1
Barley	7	Ashmour-2, Qa'a Al-Haql-7, Bohouth-2002, Bohouth-28, Bohouth-26, Bohouth-7 and Kawkaban-1
Sorghum	1	Jera'ah Mohasan-98
Millet	6	Murakab Zabid, Kadan-1, Kadan-2, Kadan-3, Kadan-4 and Kadan 5
Lentils	2	Dhamar-1 and Dhamar-2
Peas	2	Amran-1 and Yahsub-1
Beans	3	Dhafar-1, Dhafar-2 and Shibam-1
Mango	10	Surdoud-5, Surdoud-7, Surdoud-11, Surdoud-13, Surdoud-18, Surdoud-21, Surdoud-36, Kechener, Totapuri, and Surdoud-108
Total	**41**	

in subsequent years, there was still a notable effort to expand distribution to beneficiary farmers (Table 6).

In 2016, the General Corporation for Grain Production Improvement (PCGDP) played a significant role in boosting the production of improved seeds across several governorates, particularly focusing on wheat, and selling them at reasonable prices. The Corporation entered into agreements with numerous farmers, providing them with seeds and essential production inputs such as fertilizers and irrigation to encourage the expansion of wheat cultivation. After the harvest, the Corporation purchased the crops from the farmers, deducting the costs of the seeds and production inputs provided. The quantities distributed by the General Corporation for Grain Production Improvement increased from 52 tonnes in 2018 to 640 tonnes in 2022 (Figure 1).

Since 2015, several international organizations in Yemen have launched rapid response programmes to support farmers during the ongoing conflict. These programmes involve purchasing and distributing seeds through national partners like GSMC and AREA. The seeds are usually sourced directly from farmers, especially for crops like sorghum and millet, or from research and multiplication institutions.

Changes and trends

The results of this study revealed the main changes and latest trends in the use of PGR in Yemen during the study period (2007–2023) compared to the previous period which was covered by the first (FAO, 1996) and second (FAO, 2009) country reports of Yemen on PGRFA issued by FAO. The most important of these changes and

Table 6. Area (ha) and quantities of produced improved seeds (tonne) in Yemen (2012–2022) for wheat, sorghum and millet. Average seeding rate (kg/ha): wheat (140), sorghum (20) and millet (15). NA, data not available. Source: Prepared by the authors based on unpublished data of the GSMC, Dhamar, Yemen.

Crop	**Details**	**Year**										
		2012	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	**2022**
Wheat	Cultivated area (ha)	326	444	473	292	332	392	NA	NA	NA	NA	NA
	Production (tonne)	652	888	946	584	665	785	720	718	570	385	346
	Area covered by improved seeds (ha)	4,664	6,350	6,757	4,171	4,750	4,906	NA	NA	NA	NA	NA
Sorghum	Cultivated area (ha)	92	117	101	115	234	241	NA	NA	NA	NA	NA
	Production (tonne)	92	117	101	115	234	241	178	338	232	108.6	141.4
	Area covered by improved seeds (ha)	4,600	5,850	5,050	5,750	11,070	12,050	NA	NA	NA	NA	NA
Millet	Cultivated area (ha)	36	5	33	27	55	42	NA	NA	NA	NA	NA
	Production (tonne)	36	5	33	27	55	42	16	153	30.3	43.5	49.8
	Area covered by improved seeds (ha)	2,400	333	2,200	1,800	3,666	2,800	NA	NA	NA	NA	NA

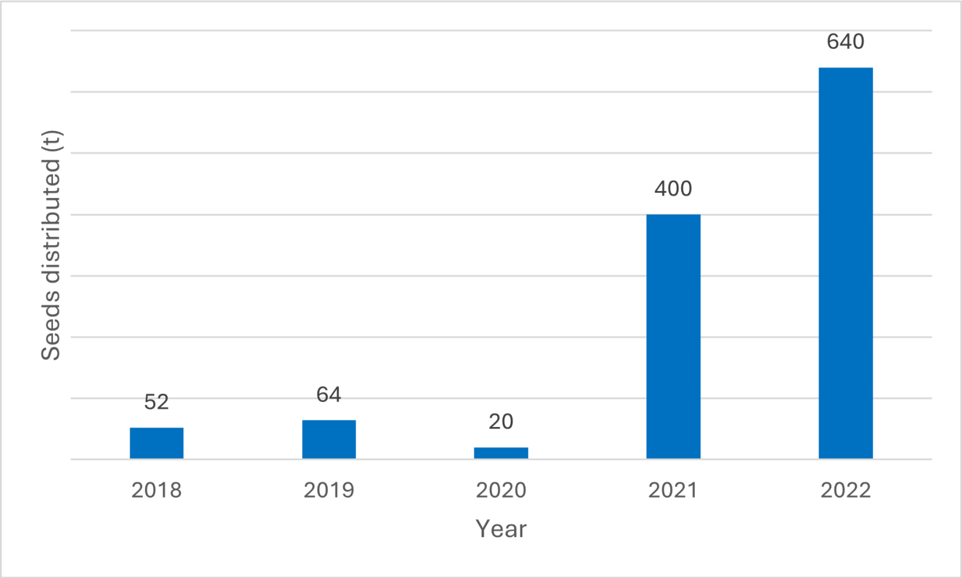

Figure 1. Quantity of wheat seeds (tonnes) distributed by the General Corporation for Grain Production Improvement (2018–2022)

trends can be summarized as follows:

- Considering the conditions of war and siege that the country has faced since the beginning of 2015, and with the growing awareness of protecting the country's sovereignty over its PGR, there has been growing interest in protecting local landraces from potential misappropriation of intellectual property rights. This defensive protection might be developed through the registration of landraces into national catalogues (Noriega, 2016). It would also be important to deposit samples in publicly accessible plant collections and document each accession with internationally recognized documentation standards, such as FAO/Bioversity International Multi-Crop Passport Descriptors (MCPD) (Alercia *et al*, 2015) and IPGRI/Bioversity crop-specific phenotypic descriptors.[1] Assignment of unique identifiers such as DOIs to accessions is also key to facilitating the exchange of germplasm passport information, ensuring that genetic resources are effectively managed and conserved. Therefore, in their crop improvement programmes, AREA prioritized the documentation of genebank accessions and registration of landraces. The Ministry of Agriculture and Irrigation also completed the steps to register many improved varieties of strategic crops. AREA submitted the required documents for sound registration procedures to the Plant Production Directorate of the Ministry, and the Higher Committee for Varieties Registration (HCRAVT) approved in its meetings the completion of their registration procedures. This included varieties of coffee, maize, wheat, barley, millet, sesame, and legumes such as lentils and peas.
- NGRC (Dhamar), in coordination and partnership with the Central Highlands Research Station (CHRS) (Dhamar) and Wadi and Al-Sahra Research Station

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[1] Full collection available at https://hdl.handle.net/10568/56589

(WSRS) (Seiyun), characterized and evaluated varieties of sorghum, wheat, barley, lentils and cowpea (58, 35, 7, 3 and 18 accessions, respectively), which were collected from the selected districts in the Governorates of Dhamar, Hadhramaut and Al-Mahra as part of the activities of the project 'Participatory Conservation and Sustainable Use of Local Landraces to Improve Farmers' Livelihoods and Their Resilience in Adapting to Climate Change in Yemen' (2019–2023). This project was funded by the ITPGRFA Benefit-Sharing Fund.

- In 2021, AREA represented by its Northern Highlands Research Station (NHRS) (Sana'a), CHRS (Dhamar) and WCRS (Al kadan), and in coordination with NGRC, implemented the largest phenotypic characterization activity – within the framework of collections – of accessions preserved in the NGRC's genebank for cereal and legumes crops. More than 1,500 accessions were characterized, as a first step towards the characterizazion of groups with distinct characteristics. The data listed in Table 7 shows the number of samples sent from NGRC's genebank to the said research stations.

Table 7. Number of samples sent by the National Genetic Resources Center (NGRC) genebank to the Research Stations (2021). CHRS, Central Highlands Research Station; NHRS, Northern Highlands Research Station; WCRS, West Coast Research Station. Source: Prepared by authors based on data from NGRC's genebank (Dhamar).

Crop	CHRS	NHRS	WCRS	Total no. of samples
Sorghum	238	218	202	658
Wheat	138	99	-	237
Barley	98	65	-	163
Lentil	40	36	-	76
Peas	40	35	-	75
Bean	43	32	-	75
Beans	53	50	-	103
Millet	-	-	97	97
Maize	-	-	33	33
Coriander	-	38	-	38
Black seeds	-	28	-	28
Total	**650**	**601**	**332**	**1,583**

- There is no doubt that the scope of distribution and use of seeds has expanded over the past years due to the work of different organizations that worked in the country and provided farmers with seeds of different crops, which is undoubtedly a positive thing. However, this expansion was not accompanied from the beginning by any coordination with AREA. This has resulted in a gap between farmers, researchers and other sectoral line authorities. Although diversity is desirable and required to enhance the resilience of farmers, the introduction of species and varieties must be based on field evaluation tests and on the results and recommendations of AREA.

- NGRC participated in implementing projects that encourage increasing on-farm diversity in plant species and within species. These projects, funded by various organizations, include the 'Agricultural Biodiversity and Climate Adaptation Project' funded by the World Bank, the 'Food Security Project', funded by the Islamic Development Bank (IsDB), the Kuwait Fund for Arab Economic Development and the State of Qatar. Additionally, the 'Participatory conservation and sustainable use of local landraces to improve the livelihood and resilience of farmers to climate change in Yemen' project, funded by FAO.

- Limited efforts to introduce quinoa as a food/fodder crop through testing trials and assessments that took place in various agricultural regions.

- The breeding programmes at AREA relied on the selection from nurseries provided to Yemen by the international agricultural research centres, such as ICARDA, through regional projects and research programmes and networks for cooperation between those centres and national research institutions. Many activities were conducted in the research stations and on farmers' fields, and these projects, programmes and networks mostly targeted agricultural crops such as wheat, barley, lentils, peas, beans, cowpea, sorghum, millet and maize. Over the past decade, the activities of most international centres in Yemen have been disrupted due to the war, siege and later the COVID-19 pandemic, making the transfer and exchange of genetic materials difficult. These challenges have persisted to the present day.

- In 2021, breeding research programmes began hybridization in vegetables (tomato) and cereal (wheat) crops. This is a new trend that must be noted and praised. It came in response to the conditions caused by war, blockade and global crises such as the COVID-19 pandemic and the Ukrainian-Russian war, and the resulting tough economic conditions, especially considering the high seed import bill from abroad. This has become a major concern among decision-makers in searching for options that encourage researchers as well as the public and private sectors to move towards local seed production. The efforts in this respect are still modest because of the lack of experience and financial capabilities.

- In this context, it is important also to point to the efforts of the Potato Seed Company in producing higher grades of potato tubers and tissue culture multiplied potato varieties in partnership with the private sector during the past five years, to reduce the import bill by producing seeds locally, as well as achieving self-sufficiency in this important food crop.

- A decrease in the number of plant breeders in agricultural research for several reasons (including retirement, death, illness and migration) in addition to the effects resulting from the war, such as the cessation and irregularity of salary payment and the absence of operational budgets.

- The process of exchanging genetic resources with the regional and international centres was halted due to the war and siege, and the paralysis or ineffectiveness of the various public service institutions.

- There have been no changes in national policies that encourage the development and trade of local varieties and underutilized crops, as the Agricultural Seeds and Fertilizers Law no. 20 of 1998 is still in place and primarily focuses on the regulation, production and marketing of adopted seeds and fertilizers, but it does not specifically address the development and trade of underutilized crops (Govt. of Yemen, 1998).

- There has been a positive and active change on the part of public and commercial sectors in the trade of local varieties and underutilized crops. This is often done with the support of external organizations within the framework of the emergency response and humanitarian aid programmes/projects, despite the parallel negative points that could accompany this endeavour. An example of this is the PCGDP establishment of a specialized market (in 2022) in the heart of the capital, Sana'a, to sell and trade types and varieties of different locally produced grains, including wheat, barley, legumes, and underutilized crops. This market complex still provides its services to both the private and public sectors. Although limited information is available about private sector activity in seed production, it can be noted that some agricultural input companies have contracted farmers to produce seeds as required by funding from international organizations working in Yemen on relief and humanitarian aid, through tenders to purchase quantities of seeds. There is also a significant activity by some companies working in propagation nurseries and producing seedlings or seeds of some crops. Examples of such companies include the 'Yemen Nabat' nursery producing tomato seedlings, as well as potato seeds using tissue culture.
- The PCGDP was established in 2016 with the aim of achieving self-sufficiency in grains. The corporation provided financial support to AREA, agricultural colleges, and seed producer associations to implement many research and production activities in addition to training and awareness raising of producers at the local community level.
- The area cultivated with improved, high-quality varieties remains limited, not exceeding 15% of the total cultivated land. Cultivation is still primarily focused on cereals and, to a lesser extent, legumes.

Gaps and challenges

This study showed that using existing PGR in NGRC's genebank and other genebanks in Yemen still suffers from many shortcomings, gaps and challenges for important aspects. Such gaps and challenges limit the process and hinder the effective use of the country's genetic resources for food and agriculture. These obstacles can be summarized as follows:

- Lack of trained and qualified technical personnel, especially in crop description and evaluation.
- Weak capabilities in using computer programmes to analyze characterization and evaluation results.
- Weak capabilities in using biotechnology, especially molecular characterization.
- Weak documentation of data and dissemination of information on characterization and evaluation.
- Research breeding programmes that promote crop diversification are limited. Although there were initial efforts to introduce quinoa, these attempts were not sustained. Yemen has a diversity of plant foods, yet agricultural research has not adequately explored these species.
- Limited use of the genetic material preserved in genebanks for plant breeding.
- Decrease in the number of plant breeders.
- Poor use of modern technologies in education.
- Weak characterization and evaluation programmes for underutilized plant species and wild food plants.
- Limited number of crop varieties released that are well adapted to local conditions, especially sorghum and millet varieties, despite the diversity and abundance of genetic material for these two crops in the national genebank.
- Weak capabilities of producing and distributing sufficient quantities of high-quality seeds of improved varieties of different crops by the government and private sectors, farmer community organizations, farmer groups and individual producers.
- Absence of a comprehensive national policy to regulate seed production and trading, whether governmental, private, commercial or non-profit.
- Weak funding for breeding, improvement and application of biotechnology programmes.
- Great weakness in encouraging and developing the trading of seeds of unexploited crop varieties.

Conclusion and recommendations

Based on the results and indicators presented above regarding the achievements, trends and challenges affecting the use of PGR in Yemen, it can be concluded that the utilization of national genetic resources remains weak and limited in contributing to food security, agriculture, the economy and sustainable development. To improve the use of these resources, several recommendations were formulated for future consideration including:

- Characterizing and evaluating local genotype groups of basic crops, underutilized crops, and wild relatives for specific and distinct traits selected according to need, importance or priority.
- Developing a documentation and information system for description and evaluation data and education programmes.
- Expanding the application of genetic characterization using biotechnology tools.
- Publishing the results of characterization and evaluation of the preserved genetic materials so that they are available to researchers, academics and those interested, and to encourage them to use and benefit from these resources.
- Issuing guides identifying the accessions preserved in genebanks.
- Strengthening staff technical capabilities in genetic characterization, evaluation and improvement through training and qualification programmes.

- Giving priority to activities related to plant breeding and providing the required financial support.
- Supporting the GSMC technically and financially to widen its coverage.
- Reviewing and developing a special law regulating the seed production and trading sector, and developing a national seed policy and strategy that defines tasks and roles of the various public and private partners.
- Increasing collaboration with international research institutes to leverage global expertise, share knowledge and access advanced technologies for the improvement of PGR.

Author contributions

Maeen Ali Al-Jarmouzi prepared the study proposal, collected, organized, analyzed the data, wrote and improved the manuscript's drafts. Khalil M. Alsharjabi contributed to reviewing and improving the study proposal, collecting the data, writing the manuscript, editing and improving the manuscript's drafts at different stages. Ahmed Amri provided support through guidance, reviewing and improving the manuscript draft, enhancing the translation of the manuscript and providing important references.

Conflict of interest statement

The authors declare no known conflicts of interest or any financial or personal relationships influencing the work or materials appearing in the article.

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Quantifying phenotypic relationships among Arsi, Bale and Jemjem cattle breeds of Ethiopia

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Abstract: Nine morphometric and 16 morphological traits were used to characterize and quantify phenotypic relationships among Arsi, Bale and Jemjem cattle breeds. A total of 441 randomly selected adult cattle (342 females and 99 males) from three purposively selected districts were used. Univariate and multivariate analysis procedures of statistical analysis software (SAS) were used to analyze the data. Clear morphological and morphometric variations were not observed among the cattle breeds. The majority of the studied cattle possessed uniform coat colour pattern (78%), black coat colour (61%), forward-oriented horns (65.8%), widely spaced horns (71.4%) and curved horns (76%). They mostly had erected humps (96.2%), small humps (66.7), mainly located at the cervicothoracic position (77.8%) with a straight face (100%) and back profile (92%) while their rump was sloppy (100%). Overall, 44.4% and 45.6% were medium- and long-tailed, respectively, while most (72.1%) of the cattle possessed medium dewlap width. Most (57.6%) of the males had medium perpetual sheaths while naval flap was not observed on most (53.2%) of their female counterparts. In addition to the univariate analysis, the multivariate analysis also failed to show significant separation among the breeds, as indicated by the short Mahalanobis distances and low eigenvalues. In conclusion, Arsi, Bale and Jemjem cattle breeds were found to be phenotypically inseparable. However, the observed phenotypic similarities among these breeds do not necessarily mean that these cattle breeds are genetically the same. Therefore, further molecular characterization is recommended to quantify the degree of genetic relationships among the studied breeds.

Keywords: Cattle, characterization, Ethiopia, indigenous breeds, morphology, morphometric, multivariate analysis

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Introduction

Ethiopian indigenous cattle genetic resources contribute significantly both to farmers' livelihoods and the country's gross domestic product (GDP) (CSA, 2021). Cattle in Ethiopia are primarily used for milk, meat and drought power. Moreover, they are a source of income and manure, as well as provide social and cultural values (Zerabruk and Vangen, 2005; Genzebu *et al*, 2012; Yimamu, 2014; Kebede *et al*, 2017; Getachew *et al*, 2020). Ethiopia has about 70.3 million heads of cattle (CSA, 2021), making them the most populous livestock species in the country. Furthermore, according to Statista (2020), Ethiopia has the largest cattle population in Africa.

To ensure that cattle production contributes sustainably to the country's food and nutrition security, proper management of the diversity of indigenous breeds is essential (FAO, 2007). To achieve this goal, Ethiopia has adopted the Global Plan of Action (GPA) for Animal Genetic Resources which has four strategic priority areas (SPAs) and 23 strategic priorities (SP) (EBI, 2016). The first SPA – characterization, inventory and risk monitoring – aims to produce sufficient and accurate information for enhanced management of animal genetic resources (AnGR). Outputs from this SPA include knowledge of the genetic diversity, population structure

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and population differentiation of indigenous breeds. To achieve these outputs, phenotypic and genetic characterization studies are required (FAO, 2012; Ajmone-Marsan *et al*, 2023).

Several cattle phenotypic and genetic characterization studies have been carried out in Ethiopia in the past three decades, leading to the registration of 28 indigenous breeds (EBI, 2016; Mustefa, 2023). The phenotypic characterization studies provided a list of the breeds believed to exist in the country, the breeds' distribution areas and characteristics, and their linear body measurements. Similarly, molecular characterization studies assessed the within- and among-breed genetic diversity and differentiation. However, the phenotypic and molecular characterization studies carried out so far have not been comprehensive, particularly in terms of breed differentiation and registration. The phenotypic studies were limited by narrow geographic coverage, inconsistent naming and varying methodologies. Molecular studies were contradictory, and showed discrepancies between phenotypic characteristics and geographical distances among the breeds (Mustefa, 2023). Moreover, some cattle breeds including Adwa, Ambo, Bale, Hamer, Jemjem, Jigjiga, and Smada were registered without adequate phenotypic characterization studies. Addressing these gaps is essential to provide a complete and country-wide picture, which in turn will inform the design of breed-specific genetic improvement and conservation programmes.

The current study targeted three registered cattle breeds: The Arsi, Bale, and Jemjem (EBI, 2016; Assefa and Hailu, 2018). Two of them, Bale and Jemjem, were not studied before while Arsi was studied by Yimamu (2014), which revealed some of the unique characteristics and distribution areas of this cattle. The breed has a compact body with a uniformly patterned black coat colour. It is reported to have originated in the Arsi highlands, with a distribution up to Bale and Sidama highlands (Assefa and Hailu, 2018). These zones were also identified as the home to other cattle breeds: Bale highland is the breeding tract of Bale cattle (Assefa and Hailu, 2018), and Sidama highland is the breeding tract of Jemjem (Sidama highland) cattle (Assefa and Hailu, 2018; Legesse and Zeleke, 2021).

The study by Legesse and Zeleke (2021) on Sidama highland cattle showed some phenotypic resemblances with Arsi. Furthermore, Legesse and Zeleke (2021) reported the neighbouring Arsi and Bale highland areas as the origin of Sidama highland cattle. Therefore, the breeds that exist in the Arsi, Bale, and Sidama highlands seem to be the same breeds with different names. Therefore, an inclusive study taking representative samples from these areas is required to quantify the level of relationships among these breeds. The current study aimed to phenotypically characterize Arsi, Bale, and Jemjem cattle breeds and quantify the level of phenotypic relationships using multivariate analysis.

Materials and methods

Study areas

The study was conducted in two regions, Oromia and Sidama. Three districts – Diksis district of Arsi zone and Goba district of Bale zone in Oromia, and Hula district in Sidama (Figure 1) – were covered. Some parameters of the sampled districts including weather conditions and agroecology are presented in Table 1.

Site and animal selection

Representative samples of Arsi, Bale and Jemjem cattle breeds were selected from their respective breeding tract. Information on their breeding tract and distribution areas were identified from previous studies (Rege and Tawa, 1999; Yimamu, 2014; Assefa and Hailu, 2018; Legesse and Zeleke, 2021). Accordingly, Diksis district was randomly selected from the highland districts of the Arsi zone to represent Arsi cattle, Goba district was randomly selected from the highland districts of the Bale zone to represent Bale cattle, while Hula district was randomly selected from the highland districts of the Sidama region to represent Jemjem cattle. Two sampling sites (Kebeles) were randomly selected from each district. Forty households that reared cattle were randomly selected from each sampling site (kebele). Within each household, the adult cattle aged four years and above were first separated from the young ones to avoid age bias. Genetically unrelated animals were separated to make the sampling representative. Then, two animals were selected randomly for the morphometric and morphological recording to avoid sampling bias. Selected animals were controlled carefully by their owners and trained labourers. Aggressive animals that could not properly stand on the flat ground were not recorded to avoid measurement bias.

Data collection

Data on morphometric (quantitative linear body measurements) and morphological (qualitative characteristics) traits were collected based on the data collection procedures described in the UN's Food and Agriculture Organization (FAO) guidelines (FAO, 2012). Data collection was performed in the morning to avoid errors regarding feeding and watering. Five researchers were involved in the data collection procedure: three handled the quantitative data while the remaining two took care of the qualitative data decision-making and recording. To reduce bias, morphometric data recording was performed by the same researcher throughout the study. Animals were measured using a centimeter-unit textile measurement tape. A total of 441 cattle (342 females and 99 males) were subjected to nine morphometric measurements (Table 2) and 16 morphological/qualitative traits (Table 3).

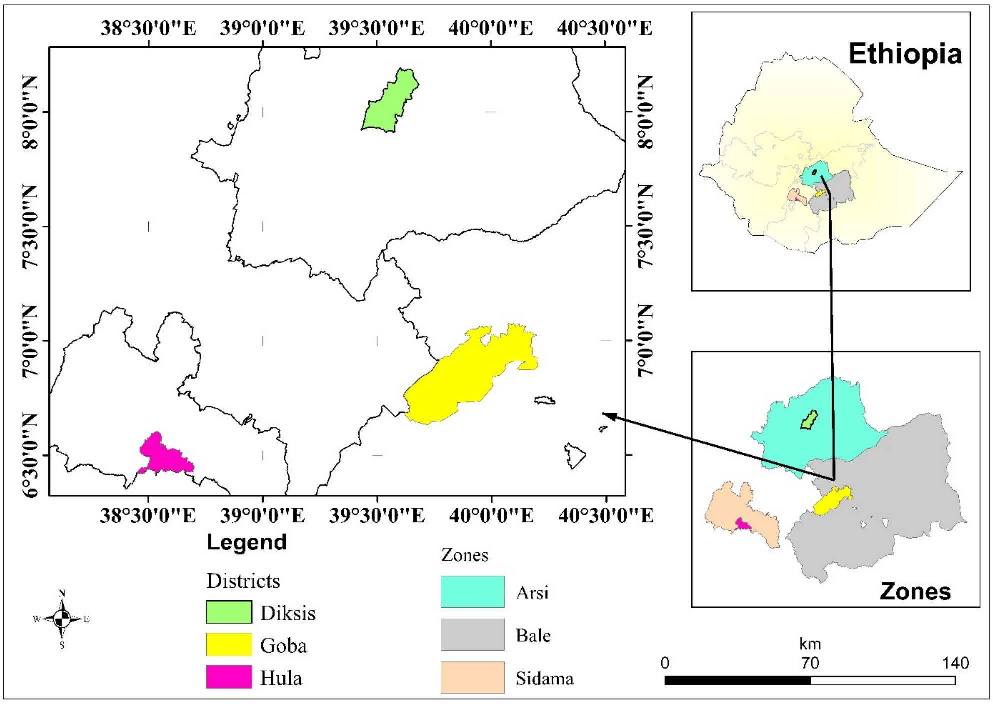

Figure 1. Map of the study areas

Table 1. Weather and agroecology-related information of the selected districts. Source: (Yimamu, 2014; Tiki *et al*, 2016; Teshale *et al*, 2017).

Parameters	Districts		
	Diksis	Goba	Hula
Altitude of the district (m.a.s.l.)	2,200–2,800	1,500–4,377	1,501–3,500
Altitude of the sampled locations (m.a.s.l.)	2,710–2,721	2,588–2,596	2,709–2,718
Temperature (°C)	18	0–23	12–22.5
Rainfall (mm)	700–1,300	1,033–1,112	1,200–1,600
Area (km^2)	283	-	270
Cattle population	139,568	-	124,472
Human population	215,337	165,712	161,214
Ethnicity	Oromo	Oromo	Sidama

Table 2. List of the linear body measurements with their definitions. These measurements were carried out using a centimetre (cm) unit measuring tape. Source: FAO (2012).

No.	Morphometric traits	Definitions
1	Body length	Distance from shoulder point to pin bone
2	Heart girth	Chest circumference right behind the two front legs
3	Height at withers	Distance from ground to withers of the front foot
4	Pelvic width	Distance between the two ends of the pelvic bone
5	Muzzle circumference	Perimeter of the mouth
6	Ear length	Distance from the root to the tip of the back side of the ear
7	Horn length	Outer side distance between root and tip of the horn
8	Cannon bone length	Distance between the fetlock joint (ankle) and the knee
9	Hock circumference	Perimeter of the hock bone

Data analysis

A Microsoft Office Excel worksheet was used to enter and manage data, while the overall data analysis was carried out using various procedures of the Statistical Analysis System (SAS) software 9.0 (SAS, 2002).

Univariate analysis

UNIVARIATE procedure of SAS (SAS, 2002) was used for data normality test, the frequency procedure (Chi-square test) was used for morphological (qualitative) data analysis, and the general linear model (GLM) procedure was used for morphometric (quantitative) data analysis. The following statistical analysis model was used to analyze the morphological data:

$Y_{ij} = \mu + S_i + B_j + e_{ij}$

where Y_{ij} is an observation, μ is the overall mean, S_i is the fixed effect of sex (i = male, female), B_j is the fixed effect of breed (j = Arsi, Bale, Jemjem) and e_{ij} is the random error. Quantitative data were analyzed separately for each sex by fitting breed as a class variable. Means (LSM) were separated using the adjusted Tukey-Kramer test (Tukey, 1953; Kramer, 1956).

Multivariate analysis

Stepwise discriminant analysis (SAS, 2016) was used to detect morphometric traits that better discriminate the cattle breeds, while discriminant analysis was applied to allocate individuals to known breeds and assess possibilities of misclassifications. Canonical discriminant analysis was also employed to deliver maximal separations between breeds (SAS, 2002). Graphic interpretation of breed differences was plotted using the scored canonical variables. Pairwise Mahalanobis distances between breeds were computed as $D^2(i|j) = (x_i - x_j)' cov^{-1}\ (x_i - x_j)$. Where $D^2(i|j)$ is the distance between breeds i and j, cov^{-1} is the inverse of the covariance matrix of measured variables, x_i and x_j are the means of variables in the i^{th} and j^{th} breeds.

Results

Qualitative characteristics

Figure 2 shows the coat colour distribution across the two sexes and three breeds studied. While coat colour was not significantly influenced by the animals' sex (chi-square value 4.5, p = 0.3480), it did vary by breed (chi-square value 37.9, $p < 0.0001$). Black coat colour was predominant across all three breeds, while black + white coat animals were observed more frequently in Jemjem cattle. Black + white coloured animals are those with predominantly black coat colour with some white patches, spots or shades. Their coat colour pattern can be also indicated as pied, spotty or shaded. The same applied to the red + white coloured animals.

The effects of breed and sex on the qualitative characteristics of Arsi, Bale and Jemjem breeds are presented in Table 3 along with the respective chi-square values and levels of significance. Sex affected 6 out of the 13 traits while breed significantly affected 7 out of the 15 traits. The majority of the studied cattle had forward-oriented (65.8%), widely spaced (71.4%) and curved horns (76%). They also mainly had small (66.7%), erected (96.2%) humps located at the cervicothoracic position (77.8%). All (100%) of the studied cattle had a straight face and a back profile as well as a sloppy rump. Medium (44.4%) or long (45.6%) tails were equally common and medium dewlap width were observed on most (72.1%) of the cattle. On the other hand, 57.6% of the males had medium perpetual sheath while naval flap was not observed on 53.2% of their female counterparts. A uniform coat colour pattern was observed on most (78%) of the cattle while all of them (100%) had straight-edged ears (Figure 3).

Comparing sexes, laterally oriented straight horns were more frequently observed in males than females. The majority of females had an erect hump while some males had a dropping hump. Males also had larger humps located at the thoracic position while females possessed small humps located at the cervicothoracic position. Comparing breeds, a higher proportion of narrow horn spacing was observed in Jemjem cattle. However, no significant differences were observed among the cattle breeds in terms of most of the qualitative characteristics.

Morphometric traits

Means (least squares), standard errors and pairwise comparisons showing the effect of breed on the morphometric traits of the studied male and female cattle populations are presented in Table 4. Relative differences among breeds were observed more in females than males. Within females, Bale cows had the largest body length (101.6cm), heart girth (139.4cm) and hock circumference (28.8cm). The Arsi cows had the smallest body length (97.1cm), pelvic width (29.5cm) and muzzle circumference (36.1cm) while their horns were the longest (22.6cm). The Jemjem cows had relatively intermediate measurements for most of the traits including body length (99.7cm), pelvic width (30.5cm) and hock circumference (27.6cm). Similarly, the Bale males had the largest heart girth (150.5cm) and hock circumference (30.7cm).

Multivariate analysis

Stepwise discriminant analysis

All nine morphometric traits were used in discriminating the females while only six morphometric traits were used to discriminate the males. The three most important morphometric variables used in discriminating the cattle breeds were heart girth, muzzle circumference and horn length among females, and heart girth, horn length and pelvic width among males (Table 5). However, low partial R-Square and F-values were observed.

Figure 2. Distribution of coat colour among three cattle breeds and separated by sex.

Figure 3. A, Arsi cattle; B, Bale cattle; C, Jemjem cattle. Female individuals on the left, male individuals on the right.

Table 3. Percentage distributions of qualitative characteristics of cattle populations by sex and breed. χ^2, chi-square; P, chi-square probabilities; NS, not significant; NA, not available; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.0001$

Qualitative traits		Breed					Sex				Overall mean
		Arsi	Bale	Jemjem	χ^2 value	P	Male	Female	χ^2 value	P	
Number of animals		152	137	152			99	342			
Horn spacing	Narrow	13.2	24.1	48.0	47.2	***	29.3	28.4	0.03	NS	28.6
	Wide	86.8	75.9	52.0			70.7	71.6			71.4
Horn shape	Straight	17.1	32.1	23.7	8.9	*	47.5	17.3	38.4	***	24.0
	Curved	82.9	67.9	76.3			52.5	82.7			76.0
Horn orientation	Lateral	17.1	29.9	22.4	27.0	**	45.5	16.4	44.2	***	22.9
	Upright	19.1	5.1	5.9			14.1	9.0			10.2
	Forward	63.8	62.8	70.4			40.4	73.1			65.8
	Dropping	0	2.2	1.3			0	1.5			1.1
Colour pattern	Uniform	82.9	85.4	66.5	29.6	***	81.8	76.9	1.3	NS	78.0
	Spotty	1.3	0.7	7.2			2.0	3.5			3.2
	Pied	11.8	8.8	23.7			13.2	15.5			15.0
	Shaded	4.0	5.1	2.6			3.0	4.1			3.8
Coat colour	Black	63.2	61.3	58.5	37.9	***	61.6	60.8	4.5	NS	61.0
	Red	21.0	24.1	8.6			21.2	16.7			17.7
	Black + white	9.2	11.7	29.6			16.2	17.3			17.0
	Grey	2.6	2.2	2			1.0	2.6			2.3
	Red + white	4	0.7	1.3			0	2.6			2
Ear shape	Straight edged	100	100	100	NA	NS	100	100	NA	NS	100
Hump shape	Erect	94.7	96.3	97.4	1.4	NS	82.8	100	61.1	***	96.2
	Dropping	5.3	3.7	2.6			17.2	0			3.8
Hump size	Small	72.4	59.8	67.1	12.9	*	9.1	83.3	224.4	***	66.7
	Medium	19.7	29.2	30.3			59.6	16.7			26.3
	Large	7.9	11.0	2.6			31.3	0			7.0
Hump position	Thoracic	25.7	24.8	16.5	4.5	NS	80.8	5.3	253.5	***	22.2
	Cervico-thoracic	74.3	75.2	83.5			19.2	94.7			77.8
Face profile	Straight	100	100	100	NA	NS	100	100	NA	NS	100
Back profile	Straight	92.8	92.0	91.5	0.18	NS	91.9	92.1	0.004	NS	92.0
	Curved	7.2	8.0	8.5			8.1	7.9			8.0
Rump profile	Sloppy	100	100	100	NA	NS	100	100	NA	NS	100
Tail length	Short	13.2	5.1	11.2	9.6	*	9.1	10.2	0.85	NS	10.0
	Medium	45.4	40.2	47.4			48.5	43.3			44.4
	Long	41.4	54.7	41.4			42.4	46.5			45.6
Dewlap width	Small	5.9	2.9	5.2	16.0	**	0	6.1	82.3	***	4.8
	Medium	69.1	65.0	81.6			43.4	80.4			72.1
	Large	25.0	32.1	13.2			56.6	13.5			23.1
Naval flap width	Absent	58.9	53.8	48.2	7.3	NS	-	53.2	NA	NA	53.2
	Small	28.6	33.3	36.5			-	33.1			33.1
	Medium	11.6	7.5	12.4			-	10.8			10.8
	Large	0.9	5.4	2.9			-	2.9			2.9
Perpetual sheath	Small	25.0	29.6	26.7	4.8	NS	27.3	-	NA	NA	27.3
	Medium	52.5	56.8	73.3			57.6	-			57.6
	Large	22.5	13.6	0			15.1	-			15.1

Table 4. The effect of breed of the cattle on their morphometric measurements by sex. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.0001$; NS, Not significant. Means within a row bearing different superscripts are significantly different; ^{a} is given to the highest value.

Traits	**Females**			
	Arsi	**Bale**	**Jemjem**	**p**
Number	112	93	137	
Body length	97.1 ± 0.56^{c}	101.6 ± 0.61^{a}	99.7 ± 0.52^{b}	***
Heart girth	132.1 ± 0.63^{b}	139.4 ± 0.68^{a}	132.0 ± 0.58^{b}	***
Height at withers	108.9 ± 0.51^{ab}	109.4 ± 0.55^{a}	107.4 ± 0.47^{b}	*
Pelvic width	29.5 ± 0.19^{b}	30.6 ± 0.21^{a}	30.5 ± 0.18^{a}	***
Muzzle circumference	36.1 ± 0.19^{b}	37.4 ± 0.20^{a}	37.8 ± 0.17^{a}	***
Ear length	16.2 ± 0.15	15.8 ± 0.16	15.9 ± 0.13	NS
Horn length	22.6 ± 0.55^{a}	19.3 ± 0.60^{b}	16.7 ± 0.50^{c}	***
Cannon bone length	16.9 ± 0.13^{ab}	16.5 ± 0.15^{b}	17.1 ± 0.12^{a}	**
Hock circumference	27.4 ± 0.16^{b}	28.8 ± 0.18^{a}	27.6 ± 0.15^{b}	***

	Males			
	Arsi	**Bale**	**Jemjem**	**p**
Number	40	44	15	
Body length	105.2 ± 1.03	108.1 ± 1.04	105.1 ± 1.70	NS
Heart girth	144.7 ± 1.25^{b}	150.5 ± 1.26^{a}	140.7 ± 2.04^{b}	***
Height at withers	115.3 ± 0.98^{a}	115.6 ± 0.99^{a}	110.7 ± 1.61^{b}	*
Pelvic width	30.0 ± 0.37	30.3 ± 0.37	29.7 ± 0.60	NS
Muzzle circumference	39.2 ± 0.33^{b}	40.3 ± 0.34^{a}	39.6 ± 0.55^{ab}	*
Ear length	16.4 ± 0.22^{a}	15.8 ± 0.22^{b}	15.8 ± 0.36^{ab}	*
Horn length	23.8 ± 1.25^{a}	24.4 ± 1.26^{a}	15.3 ± 2.05^{b}	**
Cannon bone length	17.5 ± 0.24^{a}	16.7 ± 0.24^{b}	17.4 ± 0.39^{ab}	*
Hock circumference	29.6 ± 0.30^{b}	30.7 ± 0.31^{a}	28.8 ± 0.50^{b}	**

Table 5. Order of traits used in discriminating the cattle populations from different breeds.

Sex	**Step**	**Variables entered**	**Partial R-Square**	**F value**	**Pr > F**	**Wilks' Lambda**	**Pr < Lambda**
Females	1	Heart girth	0.1832	38.01	< 0.0001	0.8168	< 0.0001
	2	Muzzle circumference	0.1394	27.31	< 0.0001	0.7029	< 0.0001
	3	Horn length	0.1928	40.25	< 0.0001	0.5674	< 0.0001
	4	Pelvic width	0.0752	13.66	< 0.0001	0.5247	< 0.0001
	5	Canon bone length	0.0583	10.37	< 0.0001	0.4941	< 0.0001
	6	Ear length	0.0307	5.29	0.0055	0.4789	< 0.0001
	7	Hock circumference	0.0278	4.76	0.0091	0.4656	< 0.0001
	8	Body length	0.0201	3.41	0.0341	0.4562	< 0.0001
	9	Height at withers	0.0189	3.19	0.0425	0.4476	< 0.0001
Males	1	Heart girth	0.2543	16.37	< 0.0001	0.7456	< 0.0001
	2	Horn length	0.1577	8.90	0.0003	0.6280	< 0.0001
	3	Pelvic width	0.0900	4.65	0.0119	0.5715	< 0.0001
	4	Canon bone length	0.0974	5.02	0.0085	0.5158	< 0.0001
	5	Height at withers	0.0568	2.77	0.0677	0.4865	< 0.0001
	6	Ear length	0.0422	2.01	0.1404	0.4659	< 0.0001

Discriminant analysis

Results of the discriminant analysis show moderate classification (65.83%) of individual animals into their corresponding breed with an error rate of 34.17% (Table 6). The highest classification into their respective breed was observed in Arsi cows while the lowest classification was observed in Arsi males. o

Canonical discriminant analysis

Multivariate statistics including eigenvalues using the first and the second canonical structures (Can 1 and Can 2) are shown in Table 7. In classifying the cattle breeds, Can 1 had a higher proportion for females (0.6066) and males (0.7876) than Can 2. However, the lowest eigenvalues were observed for both canonical structures under both sexes.

Pairwise Mahalanobis distances between the breeds studied are presented in Table 8. The shortest and the longest distances were observed among males. The shortest distance (1.77) was observed between Arsi and Bale males while Bale and Jemjem oxen were related distantly (7.31). The overall results showed the lowest and non-significant distances among Arsi, Bale and Jemjem cattle breeds.

A plot of Can 1 and Can 2 showing the maximum separation among the cattle breeds is presented in Figure 4. In line with the result of the Mahalanobis distances, females were separated less than males. Accordingly, Arsi, Bale and Jemjem cows were inseparable and categorized in the same group while relative separation was observed between Arsi and Jemjem cows. Similarly, a relative separation between Bale and Jemjem oxen was also observed.

Discussion

Qualitative characteristics

Due to their easily observable nature, unique qualitative characteristics can be used for breed differentiation. Alongside other morphometric and morphological traits, similarities in coat colour and coat colour pattern among breeds may indicated genetic similarity (Getachew *et al*, 2014; Mustefa *et al*, 2024). According to Getachew *et al* (2014), the majority (73.62%) of Ogaden cattle exhibited a uniform body colour pattern, with most (69.33%) having a grey coat colour. Similarly, Mustefa *et al* (2024) suggested that the Guraghe and Jimma cattle populations might belong to the same breed based on their phenotypic similarities. They reported that 66% of Guraghe and 77% of Jimma cattle populations had a uniform coat colour pattern with 55% of Guraghe and 65% of Jimma cattle populations having a red coat (Mustefa *et al*, 2024). In line with these results, the cattle breeds examined in the current study – Arsi, Bale and Jemjem – shared similarities in both coat colour and coat colour patterns. These phenotypic similarities suggest a potential genetic link between these breeds. However, contrasting reports from Mustefa *et al* (2023) on Harar cattle, which displayed a diverse range of coat colours and patterns, highlight the need for further molecular characterization to confirm the results of the phenotypic study.

The dominantly black coat colour and uniform body colour pattern observed in this study are in line with the results of Yimamu (2014) on Arsi cattle. The dominance of dark colours over light colours might be associated with the highland environment (Titto *et al*, 2016), since animals with darker coats are better adapted to cold conditions by absorbing more heat than lighter colour coats (Titto *et al*, 2016). Moreover, the dominantly observed black coat colour might also be associated with farmers' preferences and selection criteria as black was favoured in the studied areas.

Beyond coat colour, similarities in other qualitative characteristics were also observed. The resemblance in horn, hump, tail, dewlap, naval flap and perpetual sheath besides their perfect match in the face, back and rump profiles among Arsi, Bale and Jemjem challenge their classifications as different breeds. The slight differences noted can be taken as within-breed differences. Such differences in cattle sampled from different locations were reported by Terefe *et al* (2015) in Mursi cattle and Mustefa *et al* (2021) in Raya cattle.

Morphometric traits

Results of morphometric traits, alongside qualitative traits, can provide reliable information for quantifying the degree of relationships among breeds. In this study, the observed qualitative similarities among Arsi, Bale and Jemjem were also supported by quantitative measurements. Significant differences in morphometric measurements that would indicate distinct breeds were not observed. This was also in line with Mustefa *et al* (2024), who reported comparable morphometric measurements between Guraghe and Jimma cattle populations suggesting they belong to the same breed.

As noted in the qualitative analysis, the differences observed among the three cattle breeds might be due to within-breed variation (Mustefa *et al*, 2024). Bale cows seem to be the largest, with higher measurement values for body length, heart girth and hock circumference. Intermediate measurement values were observed in Jemjem cows, while Arsi cows were the smallest, with lower values for body length, pelvic width and muzzle circumference although they possessed the longest horns. However, Yimamu (2014) reported relatively higher measurements for body length, heart girth and height at withers for Arsi cattle in the same study area.

When compared to other Ethiopian breeds, the morphometric values of Arsi, Bale and Jemjem cattle were lower than Afar cattle (Tadesse *et al*, 2008), Begait cattle (Ftiwi, 2015), Begaria cattle (Getachew *et al*, 2020), Fogera cattle (Girma *et al*, 2016), Gojjam Highland cattle (Getachew and Ayalew, 2014), Harar cattle (Mustefa, 2023), Kereyu cattle (Nigatu and Tadesse, 2020), Nuer cattle (Minuye *et al*, 2018), Ogaden cattle (Mustefa *et al*, 2023) and Raya cattle (Mustefa *et al*, 2021). On the other

Table 6. Number and (percentage) of observations classified into breed based on discriminant analysis of morphometric traits. The diagonal bold values indicate the correct classifications of sampled animals into their respective breed.

Sex	From breed	Arsi	Bale	Jemjem	Total
Females	Arsi	**77 (68.75)**	20 (17.86)	15 (13.39)	112 (100)
	Bale	18 (19.35)	**60 (64.52)**	15 (16.13)	93 (100)
	Jemjem	24 (17.52)	25 (18.25)	**88 (64.23)**	137 (100)
	Error rate	0.3125	0.3548	0.3577	**0.3417**
Males	Arsi	**23 (57.50)**	9 (22.50)	8 (20.00)	40 (100)
	Bale	11 (25.00)	**30 (68.18)**	3 (6.82)	44 (100)
	Jemjem	2 (13.33)	3 (20.00)	**10 (66.67)**	15 (100)
	Error rate	0.4250	0.3182	0.3333	**0.3588**

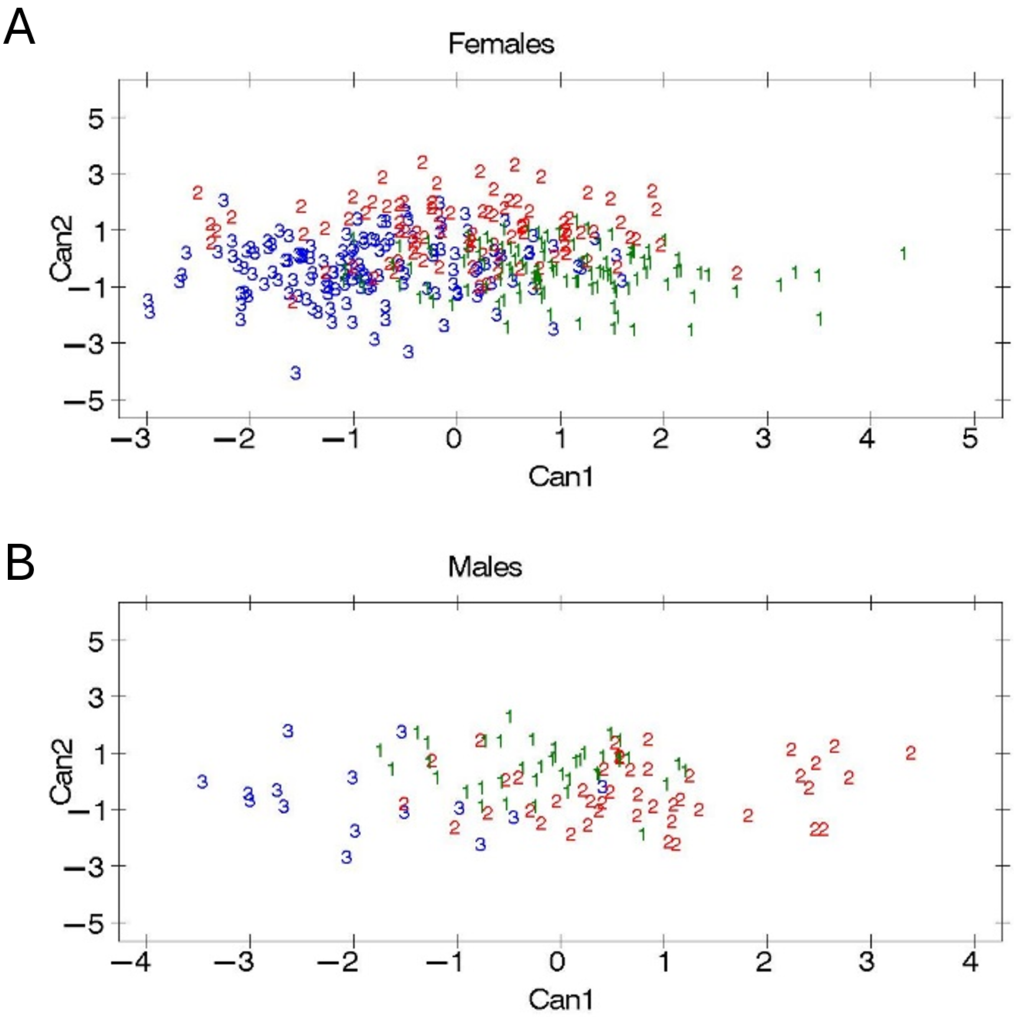

Figure 4. Plots of canonical discriminant analysis of Ethiopian cattle based on morphometric traits. A, females; B, males. Breed is indicated by numbers: 1, Arsi; 2, Bale; 3, Jemjem.

Table 7. Multivariate statistics outputs from the two canonical structures. Can, canonical structure.

	Females		Males	
Multivariate Statistics	**Can 1**	**Can 2**	**Can 1**	**Can 2**
Canonical correlation	0.6138	0.5307	0.6814	0.4353
Proportion	0.6066	0.3934	0.7876	0.2124
Eigenvalue	0.6047	0.3921	0.8670	0.2338

Table 8. Pairwise squared distances between breeds. Females above diagonal, males below diagonal.

From breed	Arsi	Bale	Jemjem
Arsi	**0**	2.88	3.32
Bale	1.77	**0**	2.72
Jemjem	4.21	7.31	**0**

hand, the morphometric values of Abergelle and Irob cattle (Zegeye *et al*, 2021) were lower than the Arsi, Bale and Jemjem. Comparable morphometric values were also reported in Arado cattle (Genzebu *et al*, 2012), Gofa cattle (Kebede *et al*, 2017), Horro cattle (Bekele, 2015) and Mursi cattle (Terefe *et al*, 2015).

Effect of sex

In most morphometric traits, males were observed to be larger than females. Such differences might be attributed to the secretion of testosterone in males, which promotes skeletal development and muscle mass growth (Baneh and Hafezian, 2009). The endocrine system plays a significant role in differentiating the two sexes, with the growth-limiting effects of estrogen being more prominent in females (Chriha and Ghadri, 2001; Baneh and Hafezian, 2009). The findings of this study are in line with the reports of Mustefa *et al* (2023) on Harar and Ogaden cattle, Mustefa *et al* (2021) on Raya cattle, and Terefe *et al* (2015) on Mursi cattle.

Multivariate analysis

Morphometric traits were identified and ranked based on their ability to differentiate between the cattle breeds. In line with the results of Mustefa *et al* (2024) on Guraghe cattle, lower partial R-Square and F-values (Table 5) were observed in the stepwise analysis, showing that morphometric traits have limited potential to discriminate the breeds into different categories. The higher the R-Square and F-values the higher the potential of the traits in differentiating the cattle breeds (Mustefa *et al*, 2023).

The higher error rate (Table 6) suggests greater shared similarities among the breeds, which reduces the chances of clearly categorizing the breeds into different clusters. On the other hand, the lower the error rate, the lower the similarities shared among the breeds. This highlights the uniqueness of each breed. The moderate classification with a considerably higher error rate (34.17%) observed in the current study, showed the presence of shared similarities among the breeds. An error rate of 1% was reported in classifying the phenotypically unrelated Harar and Ogaden cattle breeds (Mustefa *et al*, 2023).

The low eigenvalues reported for both canonical structures in both sexes (Table 7) do not support the classification of the animals into different breeds. An eigenvalue higher than 1 is accepted to approve the discrimination analysis. If the value is lower than 1, the discrimination of the studied animals into different breeds is not significant. In this study, the observed low eigenvalue disproved the presence of three breeds in the study area.

Similarly, the higher the Mahalanobis distances between breeds (Table 8) the higher the possibility of classification into different clusters. However, the Mahalanobis distances in the current study were low although Jemjem males showed relatively higher distances. This could be due to the small sample size of Jemjem oxen. The accuracy of the analysis increases with larger sample sizes. Due to the low eigenvalue ($<$ 1) in the multivariate analysis, the distances observed were not significant, supporting the conclusion that the studied cattle breeds are phenotypically inseparable.

Conclusion

According to the univariate (morphometric measurements and qualitative characteristics) as well as multivariate analysis results, the Arsi, Bale and Jemjem cattle breeds were found to be phenotypically inseparable. However, the observed phenotypic similarities among these breeds do not necessarily mean that they are genetically the same. Therefore, further molecular characterization is recommended to quantify the degree of genetic relationships among these breeds.

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Author contributions

All authors contributed to the study's conception and design. Material preparation and data collection were performed by Amine Mustefa, Awoke Melak, Hizkel Kenfo, Seble Sinke and Ahmed Abdela. Data analysis and writing the first manuscript draft were performed by Amine Mustefa. Abebe Hailu reviewed the manuscript. All authors commented on the various versions of the manuscript, and read and approved the final manuscript.

Data availability statement

The datasets generated and/or analyzed during the current study are not publicly available due to data confidentiality but are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Brazil's implementation of access and benefit-sharing and the Nagoya Protocol: Analyzing some trends and positions in the ongoing debate

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Abstract: Access and benefit-sharing (ABS) arising from the utilization of biodiversity's genetic resources and traditional knowledge is the third objective of the Convention on Biological Diversity (CBD). Since its inception, some of the parties to the CBD have enacted ABS-national legislation and in 2014, the Nagoya Protocol came into force, providing a global standard among ABS systems. Given this, Brazil has been working to implement ABS since 2001, especially after the enactment of the national Biodiversity Law (Law 13.123/2015), which is the domestic law for the Nagoya Protocol implementation. This paper examines how the implementation of ABS and the Nagoya Protocol is viewed, discussed and debated by some stakeholders. Based on qualitative semi-structured interviews, press releases, public declarations, legislation and grey literature, the paper reveals that although ABS has faced strong criticism and delivered modest results, most stakeholders consider it strategic and important, especially in the face of the bioeconomy–biodiversity nexus. In general, positions on the implementation of ABS policies and the Nagoya Protocol in Brazil can be devised in the following categories: 1) acceptance and optimistic appreciation of ABS, 2) acceptance of ABS mechanisms but impending need for adjustments, 3) acceptance of ABS mechanisms as a 'bad with it, worse without it' scenario, and 4) rejection of ABS. Our research also shows that when it comes to ABS and providers of genetic resources, debates centred on the topic of biopiracy have declined, while debates characterized by compromise, institutionalization and the steering of ABS via the implementation process are on the rise.

Keywords: ABS, Nagoya Protocol, biodiversity, implementation, Brazil, CBD, bioeconomy

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Introduction: Access and benefit-sharing and Brazil

Brazil is a global environmental superpower, and how this megadiverse nation implements its policy on access and benefit-sharing (ABS), along with the Nagoya Protocol (NP), has far-reaching implications for the entire world. As both a major user and provider of genetic resources, Brazil's ABS landscape is shaped by a dynamic industrial and agricultural sector, a vibrant academic community, and strong leadership from Indigenous and local communities. Few countries offer such a comprehensive microcosm of the challenges and opportunities surrounding the future of the Nagoya Protocol and domestic ABS systems.

Access and benefit-sharing has been a pivotal concept that marks the transition from an age in which biological and genetic resources (GR) were regarded as a common heritage of mankind toward an international system based on the sovereignty of national states. Emboldened by the Convention on Biological Diversity (CBD) (UNEP, 1992), opened for signature during the Earth Summit in Rio de Janeiro in 1992 (entered into force in 1993), parties have started to formulate ABS systems aiming to tackle different goals in the face of global inequalities. Especially in the so-called Global South, this included safeguarding genetic biodiversity by adopting CBD's "mandate of justice (distributional, procedural, and

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recognitional)” (Suiseeya, 2014) in the face of unequal technological capacities between poor and rich countries as well as the establishment of ABS-procedures for the compensation of Indigenous peoples and traditional communities’ knowledge. Last but not least, ABS was to curb potential and actual biopiracy (Rabitz, 2015). In fact, since the 1980s, researchers and activists have used the term ‘biopiracy’ to criticize the misappropriation and commodification of Indigenous peoples and local communities’ (IPLC) knowledge of seeds or plants by biotechnology companies, mainly from the Global North (RAFI, HSCA, 1998; Shiva, 2007; Robinson, 2010). The principle of national sovereignty over GR was enshrined in Article 3 and Article 15 of the CBD and was also extended to GR (excluding human genetics) (UNEP, 1992).

Access and benefit-sharing is based on Article 15 of the CBD. The latter was reinforced by the legally binding NP which was agreed in 2010 (CBD, 2011) and came into force in 2014. The NP provides the framework for national ABS regulations to be respected by third parties, especially in the event that GR are utilized outside the provider country. Broadly speaking, this is the most encompassing global ABS mechanism that applies to GR of biodiversity.

However, the ABS concept was criticized from the outset. Environmental activists, scientists and Indigenous peoples feared that private forms of ownership would be imposed on IPLC and that expropriation dynamics would be accelerated (Shiva, 2004). Contrary to the high expectations of megadiverse countries and of the NP architects, on the one hand, and consistent with the concerns and hopes of activists and IPLC, on the other, the NP has not led to significant compensation payments (Laird *et al*, 2020). The reasons for this are manifold, and both users and providers of GR have reasons to complain: a lack of global sanction mechanisms and the possibility of circumvention (Rabitz, 2015; Halewood *et al*, 2023); legal uncertainties on concepts such as access to and use of GR at the national level (Vogel *et al*, 2018) which have resulted in limitations to international trade and research (Braun, 2024); unresolved questions regarding transboundary traditional knowledge (TK) associated with GR as well as procedures to facilitate traceability (Dutfield, 2015); structural conflicts and lack of trust between IPLC and state institutions (Hayden, 2003); the ambiguity of the concept of GR, both at the global and national levels (Aubertin and Filoche, 2011; Müller, 2018). On the private sector side, non-harmonized implementation of the NP tend to expose companies to high risks (Michiels *et al*, 2022) and, last but not least, biotechnological innovations such as the new genomics tools, artificial intelligence and digital sequence information on genetic resources (DSI) pose further challenges to the successful implementation of the NP as well as of the national ABS policies (FDCL, GeN, 2022).

In Brazil, the discussion on ABS and NP covers multifaceted aspects. In general, questions of justice and political constellations (Dallagnol *et al*, 2016; Feres *et al*, 2019), descriptive aspects of legislation (Silva and Oliveira, 2018), sectorial analysis (ABIHPEC, 2017; Costa, 2017; Marinello, 2020), state transformations (Eimer and Donadelli, 2022), and comparative analyses of the legal situation (CNI, 2017) have framed the debate. The private sector, consultancies, and third-sector organizations have been especially vocal in producing guidance for ABS implementation. Likewise, influential academics such as the brothers Carlos and Ismael Nobre (Nobre and Nobre, 2019) and Ricardo Abramovay (Abramovay, 2020) have urged for the implementation of ABS in the Amazon, seeing it as having the potential to represent a major breakthrough in promoting the Brazilian bioeconomy.

The topic of implementation in particular has very recently gained momentum as the expectation of the ratification of the NP and its promulgation mounted and these then finally took place in 2021 and 2023, respectively. On the other hand, the implementation of national ABS policies and the NP were hampered by COVID-19 pandemics and unfavourable political conditions during Jair Bolsonaro’s presidential (2019–2023) term (Eimer and Donadelli, 2022).

Worldwide, the literature on the implementation of ABS systems has become more and more abundant as countries, blocs (e.g. the EU), scholars, scientists and activists have increasingly published their experiences and views (Coolsaet, 2015; Vanheusden and Van Den Berghe, 2017; Greiber, 2019; Kamau, 2019; Friso *et al*, 2020; Kamau, 2022). In Brazil, scientific interest in the implementation of ABS policies mostly emerged in light of the ratification of the NP (Silva, 2019; Ferreira, 2020; Mozini, 2020; Silva *et al*, 2021; Eimer and Donadelli, 2022). Previous studies do exist, but they are either highly technical (Rabitz, 2015; Davis *et al*, 2016) or present purely national perspectives (Segundo *et al*, 2018). Furthermore, the research conducted on the Brazilian case has also been dominated by legal analyses (Ferreira and Moraes, 2013; Davis *et al*, 2016; Ferreira, 2020; Mozini, 2020).

In this paper, I explore Brazil’s effort to implement ABS. Brazil is particularly well-suited for this qualitative case study because it is one of the most passionate advocates of the NP and a megadiverse nation, being both a provider and user of GR. Moreover, the country has been regulating its genetic heritage since 2001 (Brasil, 2001) and did not wait for the NP (ratification of the NP by Brazil did not occur until 2021 (Brasil, 2021b)) and promulgation only late 2023 (Brasil, 2023c) to develop its own ABS system. In addition to this, Brazilian national legislation in the spirit of the NP was enacted in 2015-2016 and the Ministry of the Environment has increasingly incorporated an institutional framework for ABS. As one of the first examples of ABS implementation, Brazil has attracted considerable international attention. Finally, Brazil has become an important player in the emerging bioeconomy (Backhouse *et al*, 2021) with direct influence on how ABS and NP are currently understood (Queiroz-Stein *et al*, 2024).

The aim of this paper is to reconstruct the implementation process from 2015 to 2023 from the perspective of selected involved stakeholders. By analyzing their key positions in the debate, we will cluster and explore positions in depth that highlight current trends with potential to influence the implementation of Brazil's ABS system and the NP. Given that Brazil has a long experience with ABS, its implementation hurdles, opportunities and shortcomings may reflect the challenges or even the limitations for the global implementation of ABS policies.

The paper is structured as follows: after a short introduction to our research methods and data, I summarize the ABS mechanism, implementation process and governance in Brazil. Research on the national implementation of ABS systems has largely overlooked the interaction between these systems, public policy and public debate. Most studies have focused on implementation as a purely legal or procedural process, neglecting the complex sociological dynamics involved (Matland, 1995). Siebenhüner and Suplie (2005) made an early attempt to bridge this gap by linking ABS implementation, prior to the NP, with the concept of "institutional learning". They challenged the assumption of rationally bounded actors and extended the understanding of institutions beyond their formal structures. In the context of ABS implementation, learning occurs within a network of actors historically shaped by "strong user interests", "process facilitators", and "provider interests" (Siebenhüner and Suplie, 2005). Thus, implementation is a fluid process where trust, influence, expertise, and social capital play crucial roles in the realm of policy implementation (Montgomery, 2000). According to this literature, implementation is largely processual and happens beyond purely managerial decisions; positions and trends on ABS and the NP thereby play a major role in the implementation and shall be taken into account especially due to collegiate governance of these issues in Brazil.

Empirically, the topic of ABS and NP is managed in Brazil by a relatively small community which spans activists, Indigenous leaders, bureaucrats, (natural and social) scientists, attorneys, consultants and high-skilled employees of companies that engage with the use of biodiversity (more details in the next section). In the Brazilian case, many of them have seats at the Conselho de Patrimônio Genético Nacional (CGEN). CGEN is the national committee in charge of the management of ABS and the NP; within CGEN, experts represent their own organizations, sectors and communities. Public servants who are also experts on ABS and the NP represent their ministries and secretaries at CGEN. As said before, the community is small and lack of expertise within some groups may blur divisions and organized interests, as members of determined sectorial chambers may be instated to represent others for the sake of the quorum required for the CGEN sessions. This diffused knowledge and multi-sited aspects of representation bring additional hurdles for the investigation, corroborating the fluidity of the implementation process. Given this, interviews became the privileged tool for research. Due to the specific expertise that the topic requires and given the reduced size of the ABS community in Brazil, we consider the expert interviews method the most adequate approach; according to Meuser and Nagel (2016), expert interviews reflect a tendency for institutionalization and a position in society that permits "free spaces for the construction of reality". Originally conceived upon the realities of an industrialized society (Germany), and drawing on the topic of ABS, whose interactions with TK and IPLC are self-evident, one should ask whether such categorization is convenient in this case; we nevertheless deemed the expertise and experience of IPLC of uttermost importance and drawing on Kaiser (2012), IPLC were considered full-right experts.

To address these questions and following an intensive desk phase (analysis of grey literature such as public-ministerial and sectorial reports, Indigenous public declarations, internet homepages, legislation and current state of research we selected 12 expert interviews[1] – carried out between August 2021 and March 2023 – with key representatives of different groups of the ABS landscape in Brazil (Table 1). The interviews ranged from online sessions via Zoom (Zoom Video Communications, Inc) to in-person meetings in different Brazilian cities. Some interviews were also carried out in Montreal, Canada, during the UN Biodiversity Conference (COP15, December 2022). Following Meuser and Nagel (2016), some experts hold a dominant role (in the form of social capital) in their fields, and the selection of interviewees for this paper was mostly based on this criterion. These dominant roles are established within specific contexts in the ABS arena, and can be observed among both GR providers and stakeholders in the industrial and academic sectors (users of GR). Consultants are part of this expert landscape, too. Overall, a list of experts on ABS was created by using the 'snowball method' (based on recommendations or approaching potential interviewees via existing ones and other contacts). Recognition of ABS expertise by the interviewees led us to a shortlist in which experts were categorized according to Siebenhüner and Suplie (2005) network of actors on ABS ("strong user interests," "process facilitators," and "provider interests"). Then we selected the interviewees for this paper according to the following criteria: they represent different interest groups at CGEN and actively participate in the implementation process both of the NP and the national ABS system; in addition,

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[1] The interviews were held in Brazilian Portuguese and transcribed by a native speaker, Ms. Adriana Mastrangelo Ebecken. Email: dri.mastrangelo@gmail.com. The author is also a Brazilian Portuguese native speaker and translated excerpts of the interviews into English. The text and the excerpts were subsequently proofread by an English native speaker.

they may also either observe or facilitate implementation.

We developed the interview guideline based on a set of questions with which we sought to emphasize the interviewees' positionality, expertise, awareness and sensitivity regarding ABS. The questions included the following: "Concerning ABS, where do you stand professionally?"; "What are the biggest challenges and opportunities in the implementation of ABS and the Nagoya Protocol in Brazil and worldwide?"; and "What are the most important conflicts between users and providers of GR?".

The interviews, ranging in length from 30 to 90 minutes, were transcribed and then open-coded using the MAXQDA program (Rädiker, 2023). In the second phase of the analysis, thematic clusters were formed by comparing the positions on ABS articulated both in the interviews and the desk phase. The positions and clusters discussed here are not intended to exhaustively represent all perspectives; some also reflect historical arguments that have long been part of the controversies surrounding GR and ABS. Importantly, these clustered positions serve as tools to organize and deepen the analysis of certain qualitative trends within the broader debate. They can be understood as specific frameworks (concerning ABS and the NP) within the ongoing discussions on the biodiversity–bioeconomy nexus, as highlighted by Lima (2021), Lima and Palme (2022) and Queiroz-Stein *et al* (2024), which consider the contentious landscape surrounding the use of biodiversity in Brazil, as well as its distributive, political and ecological aspects. In this context, we emphasize the textual nature of the interviews and will explore their qualitative implications, with a particular focus on the logic of ABS.

Context: ABS implementation and governance in Brazil

The regulatory framework in which the ABS mechanism is implemented in Brazil is set down by the Lei da Biodiversidade (Law 13.123/2015, LB) (Brasil, 2015) and the subsequent national decree 8.772/2016 (Brasil, 2016) which revoked the former provisional measure Medida Provisória 2.186-16/2001 (MP). The latter was the very first national-level attempt to regulate the issue of GR in Brazil (Bensusan, 2003). Prior to the issue of the MP in 2001, the Amazonian states of Acre and Amapá had created their own state laws on ABS (Santilli, 2004) (Santilli, 2005). Irrespective of regional efforts, the MP, the LB and the national decree 8.7772/2016 nationalized ABS in the early 21st century. Interestingly enough, the Federal Constitution of Brazil (Brasil, 1988) established in article 225 the protection of genetic heritage without regulating access to it. In addition, Brazil's constitution uses the concept of genetic heritage instead of genetic resources – as proposed by the CBD in 1992. The former is defined in article 225, caput, as a "common good for the use of the people" (Brasil, 1988) and Law 13.123/2015 says in article 2 (I) "information of genetic origin" (Brasil, 2015). Regulation by public law was only established after the ratification of the CBD (Brasil, 1998), paving the way for the developments described above (Segundo *et al*, 2018).

The LB and the national decree inherited parts of the organizational structures of the former MP by reinstating and strengthening the role of the CGEN, which falls under the Ministry of the Environment and Climate Change. This body manages the National System of Genetic Resource Management and Associated Traditional Knowledge (SisGen, https://sisgen.gov.br/) digital platform in which the whole process of access, prior informed consent (PIC), mutually agreed terms (MAT), research and development (R&D), notification of products, shipments of samples, auxiliary intellectual property procedures, and other elements of compliance shall be registered. The SisGen platform is primarily an instrument for declaring access (substituting the previous mandatory authorizations issued by the Genetic Heritage Management Council, CGEN, under the MP) and management of Brazilian GR dynamics. Access to SisGen is only permitted to Brazilian researchers and institutions; foreign entities or researchers are obliged to sign a partnership agreement with Brazilian institutions (Silva, 2019).

The CGEN is a collegiate board whose administration consists of four bodies: 1) a central plenary formed by twenty counsellors (11 are members of the federal administration and the remaining nine seats are allocated to civil society representatives) (Brasil, 2016), 2) thematic councils are created by the plenary to assist in decision-making, 3) sectoral councils are established as a platform for the positions of organized groups such as scientists (Câmara Setorial da Academia), IPLC (Câmara Setorial das das Guardiãs e Guardiões da Biodiversidade) and companies (Câmara Setorial das Empresas), and 4) an executive board (led by the Secretary of Biodiversity, Forests and Animal Rights) which is in charge of managing the activities of CGEN, ensuring SisGen operates smoothly, setting the agenda for further discussions, etc.

The financial body of the LB is the Fundo Nacional de Repartição de Benefícios (Brasil, 2016) (FNRB) and like CGEN also falls under the Ministry of the Environment and Climate Change. Funds originate from the annual budget of the ministry, donations, fines charged for illegal access and use of GR, benefit-sharing, etc. The FNRB funds are used exclusively for actions and activities that benefit holders of traditional knowledge and environmental conservation. Analogous to CGEN, the FNRB is also a collegiate instance with a similar structure to CGEN. The internal regulations of the FNRB were only developed in 2022 with the Manual de Operação do Fundo Nacional para a Repartição de Benefícios (Brasil, 2022) outlining the procedures for benefit-sharing being completed in October 2023. At the time of writing (early 2024), this fund remains

Table 1. List of 12 expert interviews carried out from August 2021 to March 2023 with key representatives of different groups in the ABS landscape in Brazil

Interviewees	Occupation	Date	Place
I1	Employee at a cosmetics company	3 Mar 2023	Belém do Pará, Brazil
I2	Biologist and former member of CGEN	1 Dec 2021	Brasília (online), Brazil
I3	Environmental analyst working in the branch of cosmetics	14 Mar 2022	São Paulo (online), Brazil
I4	Representative of a national association of industries	10 Mar 2022	São Paulo (online), Brazil
I5	Leading natural scientist	22 Feb 2022	Rio de Janeiro, Brazil
I6	Leading layer and consultant	14 Mar 2022	Curitiba, Brazil
I7	Academic	14 Dec 2021	Juiz de Fora (online), Brazil
I8	CGEN member and representative of traditional community	18 Dec 2022	Montreal, Canada
I9	CGEN member and representative of an indigenous group	13 Dec 2022	Montreal, Canada
I10	Activist and member of an organization that defends indigenous rights	17 Mar 2022	Brasília, Brazil
I11	Indigenous lawyer and activist	28 Jan 2023	Porto Alegre (online), Brazil
I12	Legal scholar and activist	27 Jan 2023	Curitiba (online), Brazil

non-operational, that is, implementation has not yet occurred.

After the ratification of the NP by means of legislative decree n. 136/2020 and the ratification letter of 4 March 2021 (Brasil, 2021b), Brazil declared the LB as its domestic law for the implementation of the NP. On 27 December 2023, the NP was finally officially promulgated (Brasil, 2023c). However, Silva *et al* (2021) emphasize the urgent need for the NP and LB to be harmonized, given that Brazil is now obliged to comply with ABS legislation from other NP parties and the LB itself contradicts many of the dispositions of the NP. Questions arising from the retroactivity and temporal validity of the LB, cross-border GR, and the use of foreign GR opened a new chapter in the implementation of ABS policies in the country, posing further challenges for policymakers and society (de Souza Dias, 2022).

In terms of institutional strategies, there have been multiple sets of regulatory frameworks and national and regional strategies targeting ABS. The national strategy for intellectual property (Brasil, 2021a), the national strategy and action plan for biodiversity (Brasil, 2017a), the national strategy on science, technology, and innovation (2016–2022) Brasil (2017b), and regional initiatives such as the Plano Estadual de Bioeconomia championed by the state of Pará (Governo do Estado do Pará, 2022) and the Diretrizes para a Construção Conceitual da Bioeconomia no Amazonas are prime examples in this regard (Governo do Estado do Amazonas, 2021).

Non-governmental organizations and state-sponsored institutions are also intertwined with the launch of ABS especially in the Amazon. Institutions like the Polo Digital de Manaus (https://polodigitaldemanaus.com/), the Hub de Bioeconomia Amazônica (https://fas-amazonia.org/hub-de-bioeconomia-amazonica/), and the technological innovation clusters Arranjo Amoci (https://arranjoamoci.org/) and Arranjo Namor (https://arranjonamor.org/) in Manaus and Belém do Pará, respectively, both funded through the dispositions of the Law of Innovation (Brasil, 2004) and linked to the Federal Ministry of Science, Technology, and Innovation, are supposed to facilitate and speed up the implementation of ABS and fostering the expansion of industrial intellectual property rights (IPRs) in the Amazon.

The implementation of ABS in Brazil has gradually become part of the overarching discussion on the bioeconomy and the mainstreaming of biodiversity in national development strategies (Whitehorn *et al*, 2019). The bioeconomy and ABS generally form part of the “biotechnological vision” (Bugge *et al*, 2016; Lopes and Chiavari, 2022) in the international debate on the bioeconomy. Brazil’s IPLC have however challenged such a definition by proposing the concept of “socio-bioeconomy” (Queiroz-Stein *et al*, 2024) as outlined in the Letter from the Amazon (Amazon Socio-Biodiversity Meeting, 2021), written on 20 October 2021, as part of the position taken by the Forest Peoples Alliance (https://cnsbrasil.org/alianca-dos-povos-da-floresta/) and other organizations at COP26 in Glasgow. There are ongoing disputes on the “bioeconomy–biodiversity nexus” (Lima and Palme, 2022) in Brazil, however, the specific role of GR, the national ABS system, and the NP are clearly subordinate to the more generalist approaches on the bioeconomy, forest conservation issues, etc.

Particularly since Lula da Silva began his third presidential term, ABS has also gained momentum in mainstream politics. With Marina Silva as Minister of the Environment, the new National Secretariat for the Bioeconomy issued by Federal Decree N. 11.349/2023 (Brasil, 2023a) with a specific Department for Genetic Heritage (Brasil (2023a), Chapter II, Art. 2., II, d), 3) was established. Referring to the prospects of the bioeconomy in Brazil, Silva stressed that “reindustrialization will come from the environment and ancestral knowledge” (Relly, 2023b). In a similar vein, the government has also fostered Brazil's research capabilities on biodiversity prospecting (bioprospecting) in the Amazon region with the reorganization of the Centro de Biotecnologia da Amazônia (Melo, 2023), a research institution in charge of promoting biotechnology and thereby accelerating ABS throughout the Amazon basin. Very recently, the federal government announced the plan Nova Indústria Brasil (Brasil, 2024) with the aim of increasing the technological and sustainable use of biodiversity by 1% per year until 2033.

The country's IPLC seem also to have shifted their position on ABS in recent years, following the possibilities of the bioeconomy and adding their own perspectives. Their fears of biopiracy dominated stances on GR and TK in the early 2000s (Relly, 2023a). Open letters and declarations such as the Carta de São Luís do Maranhão paved the way for a national ABS regulation (the MP in 2001). This helped to politicize the issue, and IPLC took a firm stand regarding their demands on the protection of TK coupled with the promotion of Indigenous territorial rights. Today, CGEN has established its legitimacy among key stakeholders and has genuinely become the national forum for the issue (Castro *et al*, 2022). Critical voices do exist, especially targeting the weak dispositions for prior informed consent (PIC) of the LB. Sharp criticism or rejection of the ongoing implementation process is more commonly found on the side of IPLC, activists and their supporters. In addition, overall criticism of the ABS architecture has commonly been incorporated into the overarching topic of the bioeconomy, as indicated by the positions taken by the critical group Carta de Belém (Carta de Belém, 2022).

Nevertheless, risks to the outcomes of implementation also affect more powerful stakeholders. Due to Brazil's position as an agricultural powerhouse, whose dependence on foreign (agro)genetic resources is huge (soybean, cattle genetics, fish, etc.) and not entirely covered by Annex 1 of the FAO's International Treaty on Plant Genetic Resources for Food and Agriculture (FAO, 2009), representatives of the agrobusiness sector tend to be more skeptical about the implementation or at least are more cautious about engaging with the whole process. The delay in the ratification of the NP might also be seen as a symptom of such reluctance (Eimer and Donadelli, 2022).

As Brazil has had national ABS legislation since 2001 and recently ratified and promulged the NP, the global discussions on DSI and the corresponding dematerialization of GR have placed Brazil's ABS system and experiences in the spotlight. The literature and our findings suggest that Brazil's legal concept of GR as genetic heritage (contrary to the "material" notion agreed in the CBD) has hampered discussions on this very issue since the topic has long been covered by Lei da Biodiversidade and previous regulation (Silva and Oliveira, 2018) Aside from the definition of the Lei da Biodiversidade, actors continue to associate genetic heritage with material entities, especially plants. This understanding is dominated among IPLC, in particular, since all five biocultural protocols drafted and published in Brazil are based on plants (Andrade, 2022).

The results of the implementation are nevertheless disappointing. As stated above, the Brazilian state has not yet paid any benefits to IPLC via the FNRB. Since the state has not yet begun to implement the FNRB, we cannot attest to the full-fledged implementation of ABS in Brazil. Despite those shortcomings, we will see in the following pages that there is widespread acceptance of ABS policies, and the NP and many actors feel that it is time to seize the moment and drive the implementation processes.

Results and discussion: facts and positions on the implementation of ABS in Brazil

Exact facts and figures on the implementation of the NP and ABS in Brazil are hard to obtain inasmuch as most ABS contracts are confidential and only headers including related terms like "changes and regularization", "terms of commitment", and "non-monetary benefits" are published by CGEN online (Brasil, 2023b). A side event organized by CGEN at the COP15 in Montreal (December 2022) did, however, provide an opportunity to verify some figures. The data presented here are for the period 2001–2022 and do not differentiate the period after the LB.

SisGen registration figures

Up to December 2022, a total of 68,764 access registrations were carried out by SisGen. Of these, 56,909 (83%) referred to research on Brazilian genetic heritage. A total of 3,203 registrations only involved acccessing TK and 8,652 entries stated that both GR and TK were accessed. Thus, TK accounted for approximately 17% of the net registrations. Interestingly enough, CGEN individualized the registration of access to DSI – since DSI is covered by the Brazilian ABS legislation. Of the 68,764 net registrations at SisGen, 1,411 entries declared in silico access, of which, 336 registrations stated commercial purposes, with 1,075 registrations declaring "access activities for commercial and non-commercial use of DSI on GR". Shipping figures were also published: GR were mostly shipped to the United States (35.90%), France (14.91%), the United Kingdom (12.56%), and Germany (7.07%). Biodiversity samples or DSI were shipped from all Brazilian ecological regions, but forested biomes predominated: the Atlantic Forest (39.73%) and the Amazon (39.27%) led the statistics.

Benefit-sharing

As of 2001, 3,116 users had declared products under the modality of non-monetary benefits, while 1,789 users selected monetary benefits. The overwhelming majority, 8,859 administrative declarations of products (the trigger for benefit-sharing under LB) were exempted due to the legal specifications of the LB – presumably related to agricultural research/product development which are exempted from benefit-sharing. Whether monetary benefits were paid or not is hard to assess due to the confidentiality of ABS agreements. However, when it comes to non-monetary benefit-sharing, CGEN has published (Brasil, 2023b) recent agreements. As of early 2024, in total, CGEN registered 28 non-monetary agreements involving seven companies including the cosmetics giants Natura & Co (Avon), L'Oréal™, and O Boticário™. Of the 28 non-monetary benefits agreements, 20 addressed traditional communities such as Quilombolas, riverine communities, and other traditional communities, but no Indigenous groups appear on the list. These ABS agreements are worth around R$1,000,000 each (approx. US$200,000).

At the FNRB, CGEN amassed around US$1,250,000 to safeguard biodiversity via the payment of monetary benefits (E. Relly, personal observation). As already mentioned, the Brazilian state has not yet paid any benefits to IPLC. Notwithstanding this fact, benefits in Brazil are only billed via the GR associated with TK modality, by which IPLC and users of GR establish contractual obligations according to which the state receives a 0.5% share of the net commercialization revenues. This means that ABS only occurs between private companies and providers of GR. Companies such as Natura & Co have led the way in this regard and have therefore been seen as an efficient ABS implementor. One outstanding example in this matter is the Fundo Médio Juruá (https://institutojurua.org.br/en) established in 2017 between Natura & Co and associations of communities living along the Juruá river in the western Amazon. An employee at Natura & Co (interviewee 1, I1) told us in early March 2023 that the modality of 'GR associated with TK' is the only possibility for effective benefit-sharing in Brazil inasmuch as "we [the company] have to meet the condition that benefits will be directly transferred to the community".

Moreover, most benefits that are currently being paid to IPLC (monetary benefit-sharing) were agreed on under the previous MP and not under the current and valid LB. Even under the old MP, benefits for IPLC were very scarce. Souza *et al* (2017) estimated that between 2004 and 2013, just one ABS contract was concluded with Indigenous peoples and 61 with traditional communities. Overall, 86.4% of the total ABS contracts in this period were a result of the state's sovereignty over GR. Nor do the figures provided by CGEN in Montreal during COP15 allow us to individualize ABS under the new and valid regulation. However, as the figures collected by Souza *et al* (2017) indicate, most benefits were paid to the state.

Stakeholder positions

Given the multifaceted debate on ABS, our research has clustered some positions on the ongoing implementation of and future scenarios for ABS. In so doing, I do not claim to have covered all positions of all stakeholders. Consequently, the positions presented here acquire a normative character inasmuch as they are intended to provide a general insight into and characterize the process of implementation. They may reflect both local and global constellations, since views on nature, work, property, justice and distribution vary enormously. In general, the political economy of the CBD era is still quite relevant. Siebenhüner and Suplie (2005) seek to order the institutional learning regarding implementation and Görg (2002) idea of a "field of conflicts" on GR still applies today. Most positions reflect user, facilitator and provider interests, but topics such as biodiversity loss, IPRs, national development, far-right anti-environmentalism, and the prospect of bioeconomic transitions have produced a more blurred landscape and "convivial conversations" (Lima and Palme, 2022) between former antagonists seem to be occurring continuously.

Thus, positions can be clustered in the following categories: 1) acceptance and optimistic appreciation of ABS, 2) acceptance of ABS mechanisms but impending need for adjustments, 3) acceptance of ABS mechanisms as a 'bad with it, worse without it' scenario, and 4) rejection of ABS (Figure 1). Positions here were extracted from the transcriptions of 12 interviews and clustered qualitatively according to scholarship on contemporary biodiversity debates in Brazil provided by Lima (2021), Lima and Palme (2022) and Queiroz-Stein *et al* (2023). According to these, terminologies like "territorial rights and social objectives" and "economic growth" frame biodiversity discussions in Brazil and our research will move within this established scholarship.

Acceptance and optimistic appreciation of ABS

This cluster is mainly represented by natural scientists working both at universities and national research institutions (e.g. germplasm banks and crop genebanks) and sectorial organizations (e.g. industry, bioeconomy). This cluster can be seen as the group where users' interests dominate.

Scientists working with genetic germplasm, for instance, may see themselves as bearers of a national mission to bring TK and GR from the "public domain as collecting sites" (Hayden, 2003) and as legitimizing ABS scenarios and practices vis-à-vis the idea of enfranchisement of IPLC. A former CGEN member and biologist working in this field (I2) stated that most IPLC "neither know, nor even grasp how to value what they have". Others in industry tend to fully accept ABS mechanisms, perceiving them as a tool for harmoniously reconciling nature conservation and economic development for IPLC. Interviewee I3, who works for a major cosmetics corporation in Brazil, emphasized that:

"ABS conserves because he [an IPLC individual] collects the fruit, the leaf, he's not using it for timber. Because he could be selling that wood, you know? But instead of selling the wood, what does he sell? He sells

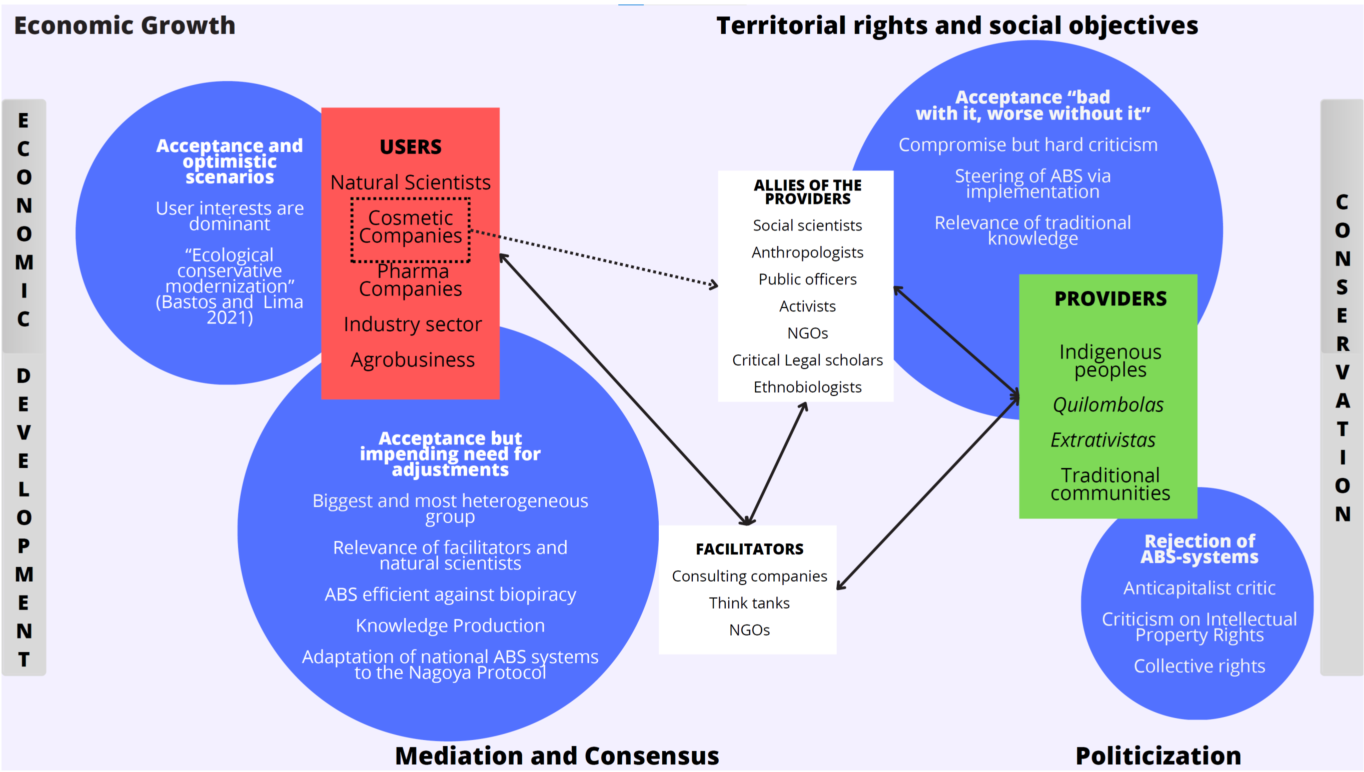

Figure 1. Clustered groups, relationships and positions among stakeholders of the Brazilian access and benefit-sharing landscape. Terminologies were developed with the help of Lima and Palme (2022) and Queiroz-Stein *et al* (2023).

the fruit, the leaf, the flower, which has a higher added value for the cosmetics industry and, in some way, he conserves it. So, for example, how much fruit do you take from the environment so that you don't have a negative impact in the sense that you manage to keep a certain amount of seeds for other trees to grow. That's management: "Oh, you need to hire a professional to do this management"; with what money? So, do through ABS!"

Positions emerging from this field tend also to be influenced by the prospects of bioinnovation and the biotechnological bioeconomy. In this realm, GR and TK are seen as a pool of resources for national development, a comparative advantage, and last but not least, a strategic asset (Nogueira, 2022). Cultural diversity seems somehow separated from biological diversity in this cluster. Another interviewee (I4), a representative of a relevant Brazilian association that fosters corporate use of national biodiversity, stressed his optimism:

"(. . .) first of all, I'm quite optimistic, okay? (. . .) Yeah, since Brazil has the greatest biodiversity in the world, right? (. . .) No country has the number of biomes that we have, right, with the reach that we have. (. . .) we do have the greatest biodiversity and (. . .) this amount of biodiversity, makes this diversity strategic for the country, right? So, the development of legislation that allows biodiversity to be exploited in a sustainable way, and that adds value to the country, is fundamental for economic development. It's a wealth that the country possesses, right?"

Positions within this group tend to minimize the conflicting issues concerning PIC and MAT or even impacts on IPLC livelihoods and cultural systems. There is a firm belief that ABS is there for good and is thus, through its market-based logic, likely to achieve the goals of national and international ABS mechanisms with regard to the utilization of biodiversity and ecological conservation. Unlike natural scientists who typically work under the umbrella of state funding, actors from industry and representatives of powerful associations in the field of agriculture and industry deplore the poor performance of the state and blame it for the inefficiency and difficulties of the whole implementation process. This cluster does not argue that existing ABS systems are flawless either, but the depiction of a win-win scenario prevails. Last but not least, this position resembles Lima (2021) "conservative ecological modernization" concept which he applied to the analysis of projects surrounding the bioeconomy in Brazil.

Acceptance of ABS mechanisms but impending need for adjustments

According to our interview record, this group is the most heterogeneous and the largest. This cluster encompasses the largest number of experts on the issue. Natural scientists, CGEN members, lawyers, compliance firms, even members of industries, agroecologists and IPLC fall into this group. People in this field can be seen mostly as facilitators who attempt to build bridges among diverging interests and possess relationships both with users and providers of genetic resources but strong user interests are also present in this group of positions. Indigenous peoples and local communities in this cluster deviate from a more critical stance toward ABS mechanisms and they are keen to identify advantages within the process of implementation.

This cluster tends to reflect on the distinctions between the LB and the NP and plead for further development of the national law, either by means of a new specific piece of legislation to address the integration of the NP into the national ABS system or by adapting implementation through guidelines created by CGEN. Another core issue for this cluster revolves around the view that ABS systems are a better outcome than the previous principle of common heritage of mankind. In general, ABS systems are deemed a game changer that represents a significant obstacle to biopiracy. That being said, scientists within this group complained about the hurdles created by the LB and the NP, and articulated the more general view that science is a common benefit to all (Bockmann *et al*, 2018). A common criticism of the LB is centred on the difficulties foreign and national scientists experience in conducting research on Brazilian biodiversity. Some within this cluster also postulated that conflicts between users and providers of GR have gradually diminished and commended the maturity and stability of the Brazilian debate on the issue. This has led to institutionalization and the effective management of the conflicts, leaving behind the more politicized discussions on biopiracy, for instance.

This last factor has significant positive consequences for the processes of implementation. Looking back at Brazil's experience with ABS so far, a leading scientist we interviewed (I5), who is an active participant in international networks, remarked:

"If we consider 2001 until now (. . .) there's been a huge maturing process, and then the new legislation [LB] came along, which has a lot of problems, but is still much better than the other one (. . .) So, I think all this has (. . .) helped reduce conflicts. Conflicts, most of the time, are not between the academy and industry, they are between the providers and industry, and even with us [scientists]. So, on this point, I'm even proud to say that in the MP we were often on the opposite side and now we're together [with IPLC] (. . .) Perhaps at the beginning there was some estrangement, I agree, but today we are very much together, defending the same things."

Knowledge production and the role of facilitators are also pivotal within this position. At the COP15 in Montreal, for example, Natura & Co, Croda International Plc and GSS Sustentabilidade e Bioinovação (a leading compliance firm in Brazil) launched the 2022 version of the Project Brogota, an outstanding piece of work, written in English, comparing the Brazilian ABS system with other national regulations around the world (GSS Sustentabilidade e Bioinovação Ltda, Croda International Plc, Natura & Co, 2022). Despite the alleged progressiveness of the ABS mechanisms (in terms of addressing justice issues), there is still a constant need for adjustments (whether that be harmonization between the NP and the LB, or working out how to meet the expectations of providers), making them very much a work in progress. Interviewee I6 who works in the compliance field also referred to these problems, affirming the many shortcomings of the implementation of ABS worldwide, the CBD's mandate of justice (Suiseeya, 2014) in addressing global disparities, and last but not least, the necessity of impending changes with the aim of creating a multilateral system for benefit-sharing:

"Now, thinking about Nagoya (. . .), not just about Brazil, about Brazilian legislation, the big issues [to be solved], whether it's Brazilian legislation or Nagoya, we have some structural problems, some conceptual problems. Because ideologically, the legislation is wonderful, the intention of the Nagoya Protocol is wonderful, the fact that the CBD, back in 1992, brought in sovereignty for countries, is also fantastic, because at that time countries needed to have sovereignty over their genetic resources, not least because of the historical legacy of colonization. So, it was absolutely necessary. The big problem we face today is that soon there will be, in a way, a war over the availability of genetic resources. Because some countries are stricter in the use of their genetic resources, others will see this as an opportunity to give away their genetic resources and facilitate access. And more than that, and this is one of the points that I'm fighting the most in relation to the Nagoya Protocol, is the overlapping of access. (. . .) so this, for me, is one of the big bottlenecks of the Nagoya Protocol, the fact that we need a multilateral mechanism, a benefit-sharing fund that facilitates or makes it possible for this to happen in a more homogeneous way and for the countries to agree to it, right?

Indigenous peoples and local communities as well as agroecologists are in a minority in this cluster. However, their positions tend to point out the double-edged nature of ABS mechanisms which range from the social valorization of TK to the dangers of cultural exploitation (Dutfield, 2015). Changes must be carried out at the state level and should address providers' interests. Natural scientists deplore the bureaucratic hurdles and their positions express the need for a more clearly defined role of basic science or for the participation of foreign scientists who wish to use Brazilian biodiversity.

Topics like PIC and MAT are more relevant and should form part of the discussion, inasmuch as they provide more legitimacy to the stabilization and management of conflicts between providers and users. In this position, we also observe "convivial conversations" with providers of GR, which do not preclude "market opportunities that may be reaped but to be open to the full spectrum of possibilities, acknowledging and accepting multiple forms of social existence" (Lima and Palme, 2022).

Acceptance of ABS mechanisms as representing a "bad with it, worse without it" scenario

In the words of a distinguished scholar of the natural sciences (I7) based in the state of Minas Gerais: "In today's context, it is bad with it [ABS], but worse without it." This "bad with it, worse without it" position has been adopted by a number of individuals predominantly from the fields of agroecology activism, environmental protection, science (especially anthropology, ethnobiology and the social sciences), law (including legal scholars), and members of governmental bodies and agencies who support or even promote the rights of IPLC and public policies. This position clearly reflects the interests of the providers of GR and builds up the other interface for "convivial conversations" (Lima and Palme, 2022), a common aspect within this position.

Members of this cluster may express strong criticism of the current ABS mechanisms and in the Brazilian case, there is a common trend of addressing sharper critics of the national ABS system due to its weaker stance on PIC (vis-à-vis the Nagoya Protocol) and IPLC's lack of political representation both in the creation of the LB (2014–2016) and in ongoing implementation in general (Guetta and Bensusan, 2018). The proximities between specific sectors of academia and IPLC' demands in Brazil was already noted by Eimer and Donadelli (2022).

Overall, the "bad with it, worse without it" position is a structural stance adopted by IPLC and their supporters in relation to ABS. It is indeed an utterly ambiguous position on the general concept of ABS, and such ambiguity has accompanied the implementation process. In Brazil at least, IPLC, NGOs and numerous grassroots organizations have nurtured a well-considered viewpoint – in the sense of the role of IPRs among IPLC as described by Ido and Valentini (2018) – on the possibilities of ABS to address or instrumentalize IPLC demands. Although "bad with it, worse without it" may imply a lack of alternatives, limited room for manoeuvre, or even rejection, this position seems to nevertheless entail a predisposition to critical compromise and the creation of institutional spaces. This position was clearly taken by the signatories subscribers (many of them from IPLC associations) of the Carta Aberta de Recomendações da Sociedade Civil Brasileira na 15ªConferência das partes da Convenção da Diversidade Biológica e seus Protocolos Terra de Direitos (2023), who stressed that: "despite all the criticism that the Biodiversity Law deserves, it also brings advances, such as the establishment of community biocultural protocols, the result of joint (...) struggle by peasants, Indigenous peoples, traditional and quilombola communities."

Ambiguity marks the complex position of Indigenous peoples in the face of the prospect of the commodification of TK and the rapprochement of market relations. In this sense, Bonifácio José Baniwa's argument, an Indigenous scholar from the Baniwa people from northern Brazil, still resonates and it is quoted as an authorized and actual perspective on TK and its relationship to markets (Alencar *et al*, 2003):

"We work with crafts that have traditional values, traditional knowledge, and traditional meanings (...) when we try to valorize these through sales, the market has its own requirements and rules. We will have to accept these rules if we want to improve the community's income, but at the same time, we may end up breaking the law of traditional knowledge. It seems that one side is stronger than the other. We're trying to work on the market understanding our way and not our knowledge having to fit the market. This is the main challenge for traditional knowledge."

In this cluster, the LB is seen as an unjust piece of legislation, a law that enabled access to instead of protection of TK. Nevertheless, implementation of the LB by means of the national decree and the establishment of the new CGEN provided IPLC with some opportunities. Our research was conducted at a rather difficult time, since Jair Bolsonaro's presidential term and the COVID-19 pandemic prevented or hindered direct participation, leaving IPLC with fewer tools to influence decisions at CGEN level, inasmuch as health and security (conflicts on land) worries became a priority. Irrespective of this, IPLC participation at CGEN is of utmost importance to tackle the unfavourable rules of the LB. Interviewee I8, CGEN member and representative of a traditional community in central-eastern Brazil stated:

"The implementation makes it possible for us to have an impact in some way. I think it's an opportunity in the sense that we can influence the agenda (...); we couldn't do this for the last four years because of Bolsonaro's term, but now, with the new government [reference to the victory of Lula da Silva in the 2022 presidential election], we have this great opportunity to make implementation really go to the grassroots."

The fact that the LB allowed the development of biocultural protocols as valid documents for trade, access and benefit-sharing is seen as a reason why the law has not been rejected outright. Interviewee I9, an Indigenous woman from northern Brazil who is also a CGEN member, stated that these protocols are "our dream (...) especially because they [the federal government of Brazil] need to foster the biocultural protocols and respect the opinion of the peoples."

The "bad with it, worse without it" position predominates in our interviews with IPLC. In this cluster, inequality, power relations, but also IPLC's leading role in the implementation and "biocultural jurisprudence" (Bavikatte and Robinson, 2011) are key. Biopiracy is still a risk for some. However, we observed a structural shift toward institutionalization with a view to changing the ABS mechanism from the inside, given that the complexity of the issue means it constantly takes on new forms, is reframed, and poses new challenges, such as DSI, transborder GR and overall harmonization with the NP. There are, however, several issues that remain unresolved by legislation. These include weak PIC dispositions, the question of unidentified TK, and the digital system enabling access through SisGen. Last but not least, ABS is seemingly expected to be a tool for territorial claims and IPLC rights. This link between ABS and territorial rights is illustrated by interviewee I10, an activist working at Conselho Indigenista Missionário and an advocate of Indigenous territorial rights, who told us that "from the moment you highlight people's coexistence with biodiversity [referring to TK] (. . .) you need to have a territory of your own."

Rejection of ABS

In Brazil, the outright rejection of ABS systems only plays a minor role in the debate. The people who argue for the rejection of the concepts underlying the implementation of ABS have long-standing critical expertise on the topic as well as excellent moral reputations. Some are seen as intellectual authorities on TK and IPLC rights. This cluster comprises Indigenous peoples and legal scholars. Their main argumentation against ABS is rooted in the issue of individual IPRs (against the background of Indigenous collective forms of property) and the encroachment of capitalist logics on IPLC's collective social structures. In addition, questions regarding PIC quickly come to the fore in this cluster.

Those positioning themselves against ABS systems generally claim that CBD-based solutions are not suited to resolving the issues concerning TK and IPLC rights. Prior informed consent has been a structural argument in this regard and discussions on how the representation of Indigenous peoples works tend to disregard ABS mechanisms. In the Brazilian debate, this position can be traced back to 2003 when the notorious Brazilian anthropologist Eduardo Viveiros de Castro questioned whether it was "the community that produces the consent or the consent that produces the community?" (Alencar *et al*, 2003). Rejecting any possibility of a stable PIC concerning access to TK, Viveiros de Castro insisted on the structural nature of unsuccessful consent, given that Indigenous communities do not operate according to the political models of liberal/Western collective representativeness. Casting doubt on the legitimacy of IPRs emanating from GR associated with TK, this view clearly prevails among those who reject ABS mechanisms. This is illustrated by interviewee I11, an Indigenous lawyer and activist from southern Brazil, who asked: "Who is the juridical person who represents my people? This person does not exist", making the case for the impossibility of a legitimate PIC under Brazilian Biodiversity Law. The issues concerning PIC in this cluster are centred on the limitations of the liberal legal tradition and criticism of capitalism. Interviewee I12, legal scholar, lawyer and long-standing activist for Indigenous rights in Brazil, noted that "the discussion on property (. . .) is an essential discussion. If you introduce private property within a system in which private property does not exist, you will destroy this system." Drawing on his own remarks, I12 went on to state his position on ABS mechanisms more clearly:

"(. . .) in any case, between not having any legislation and having legislation that regulates access, that is, that opens another door to usurpation, I would say the following: we have three forms of usurpation, right? Free usurpation: everyone goes there and does what they want, a mess; legal usurpation; and illegal usurpation, which is against the law and may be punished. Free usurpation ceases to exist when there is regulation, leaving just the other two. So, if this regulation opens up too much space for legal access, it's better not to have it. I think the regulations we have in Brazil open up a lot of space."

Conclusion

With Brazil being both a major provider and user of GR, it is one of the few countries in the world that can illustrate the true complexity of the situation to help comprehend the challenges, opportunities and perhaps even limits of the ABS mechanisms that were agreed upon at the CBD and in subsequent agreements (in our case, the NP). In this regard, Brazil can be seen as a microcosm of the (future) global ABS landscape. Notwithstanding this, ABS has not unleashed its global potential and has frustrated some expectations, especially with regard to benefits, legal uncertainties and administrative failures (here reported in the case of the Brazilian State). In this regard, Brazil has also contributed to the alleged problems implementors and COP negotiators frequently express (Silva *et al*, 2021).

Nevertheless, ABS in Brazil is robust. CGEN has gained legitimacy among stakeholders and the majority adhere to its premises, notwithstanding the justified criticism of weak PIC, high transaction costs, unequal power relations, and a lack of proper instruments of IPRs that reflect Indigenous sensitivities regarding the collective nature of their societies. We observe that the discussion on ABS mechanisms has also changed considerably and has incorporated other transversal axes such as the bioeconomy and the mainstreaming of biodiversity in national strategies (within the bioeconomy–biodiversity nexus). Politics have also shaped the spaces for debate and participation. Having undergone massive political change in the last ten years, the role of biodiversity in Brazil has been in the spotlight in a very different way. Between the tug-of-war among powerful agrarian interests and the transition to a low-carbon economy, our research revealed that the ABS and NP topics, which predate the discussions on the bioeconomy, have also been increasingly appropriated by the latter. The topic of biopiracy, for instance, which characterized the debate (especially among IPLC) during the early CBD years and the first regulation provided by the provisory measure n. 2186/2001 (2001-2015) (Brasil, 2001), for instance, have been superseded by the negotiated participation of IPLC in the national economy. In this regard, we observe increased entanglement of ABS with the prospects of a particular version of the bioeconomy, especially the biotechnological, bioecological and so-called bioeconomy of sociobiodiversity. These prospects provide opportunities but also pose risks to the actors involved. Drawing on the literature on legal anthropology, and by crossing this with the interviews, we see a trend toward compromise via the multifaceted terms of implementation, enabled by the collegiate governance of CGEN. The fact that the position that pleas for the outright rejection of ABS systems is the least relevant in the debate suggests this trend. CGEN's authority and its position as a national forum indicate that too.

Although implementation has been disappointing, that has not prevented actors from attempting to influence the course of things. Creating future scenarios and the possibility of shaping the course of implementation through CGEN governance continue to offer actors an arena for adapting national legal provisions produced by the LB as well as guiding the contingencies of the NP at the national level. In this particular regard and based on IPLC's positions on the issue of ABS, we see a major shift toward the stabilization and institutionalization of the issue. The heated debates of the past are still relevant, but IPLC are now acting pragmatically and programmatically, having even built alliances with some users and facilitators. The structural nature of the CBD arena, centred on users', facilitators' and providers' interests, still holds true but the situation has become more blurred, inasmuch as actors are compromising more. On the side of IPLC, compromise on ABS tends to address another structural claim of the post-CBD era: territorial rights as the underlying basis of an effective ABS system.

The positions raised in our study represent only a fragment of a broader and more complex implementation process. Due to the collegiate governance of ABS in Brazil, these positions tend to influence implementation. Aligning to national development and reflecting more clearly users' interests and the upper hand of natural sciences in the process, we clustered the position "acceptance and optimistic appreciation of ABS". The position "acceptance of ABS mechanisms but impending need for adjustments" seems to encompass a wider range of stakeholders with diverse backgrounds and perspectives, from those advocating prioritizing users' interests to those arguing for better protection of traditional knowledge. A more critical perspective, which leans toward providers' interests while still seeking compromise with users and facilitators (such as the State and consulting companies), is captured in the position "acceptance of ABS mechanisms as a 'bad with it, worse without it' scenario". Finally, the "rejection of ABS" position, although less influential in the debate, is rooted in historical arguments about the inadequacy of TK to be accessed through ABS. Despite ongoing debates, trends toward implementation, institutionalization and governance of GR and TK in Brazil, ABS remain highly contested.

Conflict of interest

The author declares that he has no competing interests or relationships that might have influenced the work reported in this paper.

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Checklist and prioritization of crop wild relatives in Sudan and South Sudan

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Abstract: Crop wild relatives (CWR) encompass wild plant species or subspecies closely related to domesticated crops. This study presents the first comprehensive checklist and prioritized inventory of CWR for Sudan and South Sudan. Building on the regional CWR list for Northeast Africa, we identified 499 CWR taxa belonging to 44 families, with 90% of these being native species. The most prominently represented families were Poaceae (148), Fabaceace (72) and Convolvulaceae (43), while *Panicum* (32), *Eragrostis* (27), *Ficus* (24) and *Pennisetum* (20) were the most frequent genera. A prioritized inventory of 85 CWR taxa was developed based on three criteria: economic value, utilization potential and threat status. The prioritized CWR are predominately native (78%) and encompass 12 families dominated by Poaceae (38), followed by Solanaceae (9), Fabaceae (6) and Cucurbitaceae (6). Priority genera included 27, with *Digitaria* (17), *Solanum* (9) and *Cucumis* (5) emerging as key genera for conservation attention. This comprehensive national CWR inventory provides a crucial foundation for developing targeted conservation strategies in Sudan and South Sudan.

Keywords: Crop wild relatives, conservation, checklist of crop wild relatives, priority inventory

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Introduction

Crop wild relatives (CWR) are wild ancestors of plant taxa that are closely related to domesticated crops. These wild plants possess a high reservoir of genetic diversity for improving the resilience and productivity of our cultivated crops (Ford-Lloyd *et al*, 2011). CWR possess a broader genetic diversity compared to domesticated crops because of their adaptation to various climatic conditions (Dempewolf *et al*, 2017). This genetic richness allows CWR to share valuable genes with their domesticated counterparts, enhancing crop resistance to pests, diseases and environmental stresses (Barazani *et al*, 2008). Globally, an estimated 50,000–60,000 CWR occur, with 10,740 identified as potential contributors to future food security (Maxted and Kell, 2009). Recognizing their importance, a study by Vincent *et al* (2013) established an initial global priority list of 1,392 species for conservation and utilization to ensure food security. However, CWR face numerous threats, including nitrogen deposition, land-use alterations, invasive alien species, overgrazing, urbanization and climate change (Ford-Lloyd *et al*, 2011).

Climate change is projected to have a detrimental impact on global crop production (Lobell *et al*, 2011). Over the past three decades, global warming has accelerated and is expected to intensify further in the years to come (IPCC, 2020). In Africa, the effects of climate change on food production are already apparent, manifesting as changes in rainfall patterns, rising temperatures and an increased frequency of extreme weather events (IPCC, 2020). These changes have resulted in reduced crop yields, particularly in sub-Saharan Africa (IPCC, 2020), which is home to

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approximately 45,000 plant species (Linder, 2014), and where a significant portion of the population relies on plant resources as the foundation of their diet (Gollin and Rogerson, 2014).

For the comprehensive utilization of CWR in crop improvement programmes, it is vital to conserve, classify and make them accessible to researchers and plant breeders at national, regional and global levels (Maxted *et al*, 2015). Hence, urgent measures involving complementary protection, both *in situ* (on farms) and through storage in *ex situ* facilities (genebanks), are essential steps to preserve these genetic resources and ensure their availability to researchers and breeders (Maxted and Kell, 2009). The initial phase of CWR conservation planning entails the development of a checklist, defining the taxon names of existing CWR within a specific region or country (Maxted *et al*, 1997). Subsequently, the creation of a priority CWR checklist involves reducing the number on the checklist to more manageable levels. This is achieved by applying criteria such as threat status, endemicity and potential utilization (Ford-Lloyd *et al*, 2008).

To assess the potential utilization of CWR in plant breeding, researchers rely on two main concepts: the gene pool concept (Harlan and De Wet, 1971) and the taxon group concept (Maxted *et al*, 2006). These concepts are crucial as they help us to understand the genetic relationships between cultivated crops and their wild relatives. Close relatives hold a higher likelihood of intercrossing than distant ones. The gene pool concept furnishes valuable information to plant breeders, aiding them in selecting germplasm for crossbreeding and plant improvement. It is divided into three categories: the primary gene pool (GP1), where GP1a represents the cultivated forms and GP1b the wild or weedy forms; the secondary gene pool (GP2), consisting of species that are less closely related but still capable of gene transfer, albeit with difficulty; and the tertiary gene pool (GP3), consisting of distantly related species where gene transfer is either impossible or requires advanced methods such as genetic engineering (Harlan and De Wet, 1971). In cases where gene pool concept information is unavailable, the taxon group concept serves as an alternative. The taxon group concept categorizes relationships as follows: taxon group 1a (TG1a) is the crop itself, taxon group 1b (TG1b) includes the same species as the crop, taxon group 2 (TG2) consists of species within the same series or section as the crop, taxon group 3 (TG3) refers to species in the same subgenus as the crop, taxon group 4 (TG4) includes species in the same genus as the crop, and taxon group 5 (TG5) includes species in the same tribe as the crop but belonging to a different genus (Maxted *et al*, 2006).

CWR checklists and prioritized inventories have been established in various countries worldwide, including Mexico (Contreras-Toledo *et al*, 2019), the United States (Khoury *et al*, 2013), Spain (Rubio-Teso *et al*, 2018), Portugal (Magos-Brehm *et al*, 2008), Benin (Idohou *et al*, 2013), Tunisia (Mokni *et al*, 2022), Italy (Ciancaleoni *et al*, 2021) and South Africa (Holness *et al*, 2019). A recent study by Aldow *et al* (2023) developed the first regional CWR inventory for Northeast Africa. However, there has been no prior investigation into CWR diversity in Sudan and South Sudan. Thus, the objectives of this study are 1) to prepare Sudan and South Sudan CWR checklist, and 2) annotate this checklist to prioritize it for active conservation using the Interactive Toolkit for CWR Conservation Planning (Magos *et al*, 2017).

Geographical context

Sudan and South Sudan, located in Northeast Africa between latitudes 4° and 22° N and longitudes 22° and 38° E (Zaroug, 2006), are bordered by Ethiopia and Eritrea to the east and Egypt to the north. The combined total area of both countries is approximately 2.5 million km^2, with Sudan covering about 1,878,000km^2 and South Sudan about 646,883 km^2 (Zaroug, 2006; World Bank, 2021). Pasture and forests cover 40% of the land, while cultivated area accounts for about 33% of the total area, of which only 21% is actively cultivated, resulting in very low crop yields (UNEP, 2007). The two countries are divided into five ecological zones: desert, semi-desert, woodland savannah, flood region and montane vegetation. Agriculture is the main source of income for 60–80% of the population (Elgali *et al*, 2010). The five agricultural systems include mechanized rain-fed agricultural schemes, traditional rain-fed agriculture, mechanized irrigation schemes, traditional irrigation and livestock husbandry/pastoralism (Zaroug, 2006). Approximately 90% of the farming areas in the country depend on precipitation (Siddig *et al*, 2020), while in South Sudan, despite more favourable climatic conditions, agricultural output remains low (Diao *et al*, 2012).

Materials and methods

CWR checklists and inventories are the main starting points for effective conservation strategies (Maxted *et al*, 2015). This study is based on the CWR diversity identified for Northeast Africa (Djibouti, Eritrea, Ethiopia, Sudan, and South Sudan) by Aldow *et al* (2023) to create a checklist and a priority inventory for Sudan and South Sudan. We excluded invasive species documented for Sudan and South Sudan from the Global Invasive Species Database (GISD) of IUCN (http://www.iucngisd.org/gisd/) and the Invasive Species Compendium (CABI) (http://www.cabi.org/isc/).

Our approach involved a three-step process:

1. Starting point: We began with the comprehensive CWR checklist for Northeast Africa compiled by Aldow *et al* (2023), which included 1,020 taxa.

2. Regional refinement: We then used a digitalized floristic checklist of Sudan and South Sudan, compiled by the first author based on *Plants of Sudan and South Sudan: An Annotated Checklist* (Darbyshire *et al*,

2015) during the establishment of the CWR checklist for Northeast Africa, to retain a checklist with taxa occurring only in these two countries. Note that in the flora of Sudan and South Sudan, the Compositae family is referred to as the Asteraceae family.

3. A priority inventory of CWR was selected based on three criteria:

(A) Economic value: FAOSTAT crop valuation (FAO, 2021) was used to select taxa with economic importance based on the related crop.

(B) The utilization potential: priority taxa within gene pool categories 1b, 2, 3 and taxon group categories 1b, 2, 3 and 4 (with documented use in crop development only) were selected based on the information available in the Germplasm Resources Information Network Taxonomy (USDA, 2023) and the Harlan de Wet CWR inventory (Vincent *et al*, 2013).

(C) Threat status: although (Kell *et al*, 2017) recommended incorporating threat status as a prioritization criterion, its application was limited due to the scarcity of Red List data for these countries (only 7% of plant species according to Darbyshire *et al* (2015). However, based on expert consultation, the endangered South Sudanese coffee species *Coffea neoleroyi* A. P. Davis (IUCN, 2022), was added to the priority checklist.

Additional information was incorporated into the compiled CWR checklist and inventory. This supplementary data encompassed taxonomic details such as accepted taxa names, synonyms and authorities. Additionally, it included the common names of related crops, native status, the utilization of the crop, the type of relatedness (gene pool or taxon group), confirmed or potential use in crop breeding, distribution status along with relevant references, and *in situ* and *ex situ* conservation statuses.

Results

CWR Checklist

The CWR checklist of Sudan and South Sudan contains 449 taxa (including subspecies and varieties), belonging to 88 genera across 44 families. Both native and introduced taxa are included. Invasive taxa, like *Sorghum halepense* (L.) Pers, were removed from the checklist. Sudan has 133 taxa, 59 genera, and South Sudan 161 taxa and 47 genera. Over 90% of the listed CWR are native to these two countries. The most common plant families include Poaceae (grasses) with 148 taxa, Fabaceae (legumes) with 72 taxa, and Convolvulaceae (morning glories) with 43 taxa. The genera with the highest number of CWR taxa are *Panicum* (32), *Eragrostis* (27), *Ficus* (24) and *Pennisetum* (20). Details on the distribution of taxa can be found in Supplemental Table 1 and Figure 1.

Priority checklist

The priority checklist of Sudan and South Sudan contains 85 taxa related to 12 families and 27 genera. Figure 2 illustrates the distribution of these priority taxa across both countries. The most mentioned families were Poaceae (38), Solanaceae (9), Fabaceae and Cucurbitaceae (6 each), while *Digitaria*, *Solanum*, *Cucumis* and *Echinochlo* were the most mentioned genera (Supplemental Table 2 and Figure 3).

The closest wild relatives to the crop GP1b, TG1b, TG2 and GP2 represent about 49% and GP3 counts for about half of the priority taxa (Figure 4a and Supplemental Table 3). The confirmed use of taxa is about 8%, potential use 17%, confirmed and potential use 14%, and unconfirmed use 61% (Figure 4b and Supplemental Table 3).

CWR in the checklist have provided a number of beneficial traits to crops such as chickpea, teff, coffee, finger millet, cassava, rice, cotton and sorghum found in Sudan and South Sudan (Table 1).

Discussion

The inventory conducted in Sudan and South Sudan reveals a rich diversity of native and introduced taxa associated with a broad range of crops. This provides a crucial foundation for the development of national policies and strategies in both countries. These strategies should prioritize the conservation of the identified CWR genetic diversity, both *in situ* and *ex situ*, with the ultimate goal of ensuring the long-term conservation of these valuable resources for the benefit of future generations (Kell *et al*, 2017). In light of the critical importance of CWR for global food security and sustainable agriculture, researchers and scientific institutions have advocated for a coordinated global CWR conservation strategy (Dempewolf *et al*, 2014), which should encompass both *in situ* and *ex situ* approaches for effective conservation.

The checklist of CWR identified a significantly higher diversity of CWR in South Sudan (37,9%) compared to Sudan. Interestingly, about one-third of the CWR identified are found in both countries. Additionally, nearly half (45%) of the priority CWR are shared between the two nations. Unfortunately, most documented plant taxa in these countries are outdated due to a lack of recent research, likely a result of long-standing civil conflicts. This highlights the urgent need for taxonomic experts to update the classification of these CWR.

National CWR conservation strategies should be harmonized with regional and international initiatives. As proposed by Maxted *et al* (2015), integrating national and regional CWR conservation strategies is highly beneficial. This recognizes that prioritization criteria for CWR conservation at the national level can often be aligned with the regional level. This overlap facilitates collaboration between various agencies (governmental, private, or voluntary) at both levels, leading to the development of more effective CWR conservation strategies.

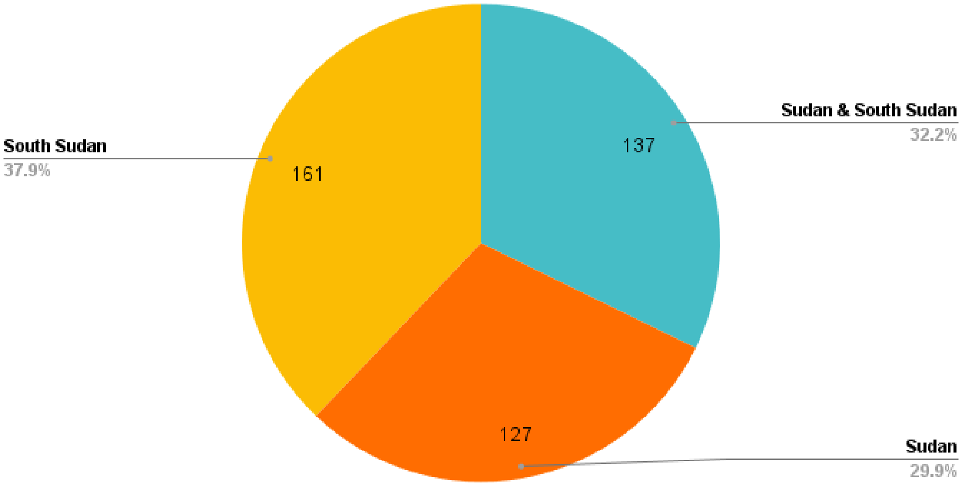

Figure 1. Distribution of CWR taxa in the checklist in Sudan and South Sudan

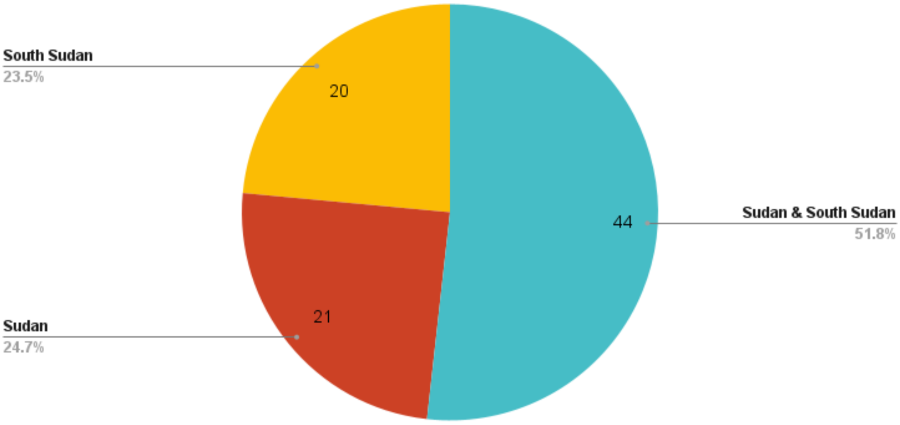

Figure 2. Distribution of priority CWR in Sudan and South Sudan

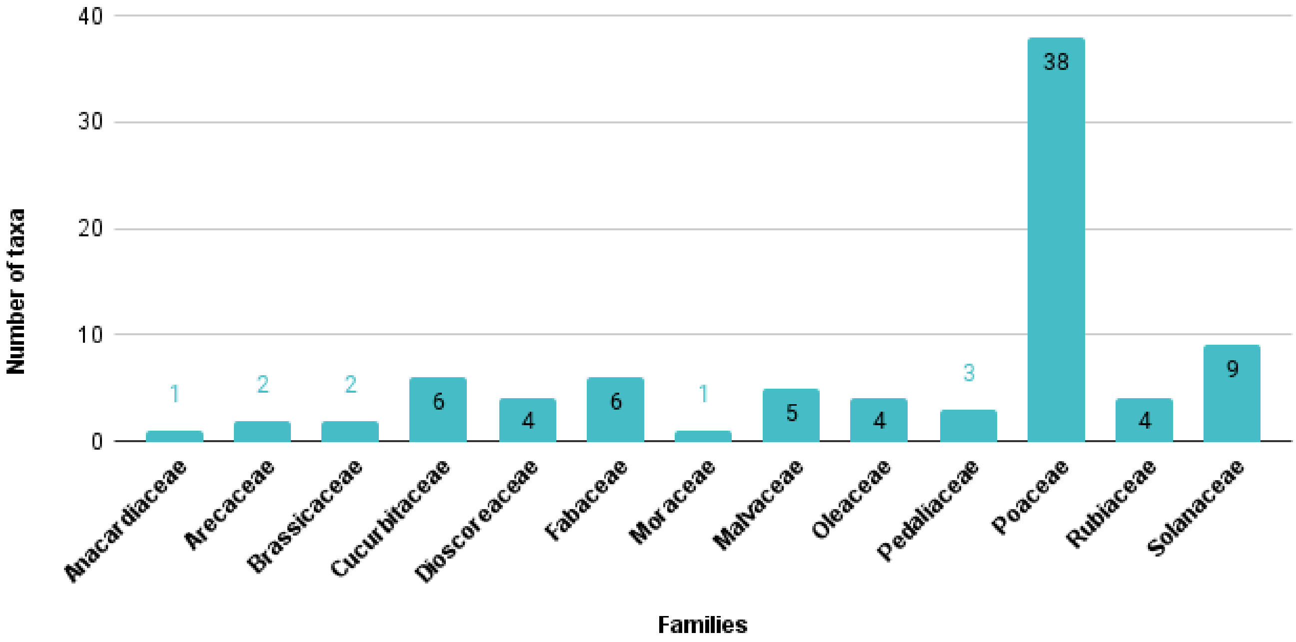

Figure 3. Diversity of taxa among CWR families within the priority CWR inventory in Sudan and South Sudan.

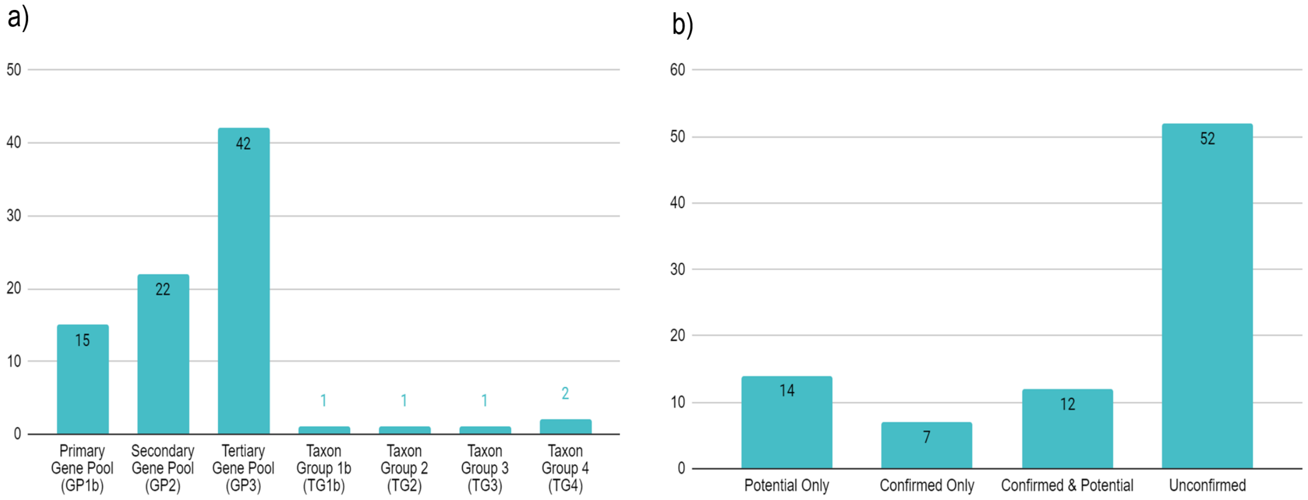

Figure 4. Genetic relatedness and use potential for priority CWR inventory in Sudan and South Sudan. a) shows the genetic relatedness of the priority CWR inventory in Sudan and South Sudan, based on the gene pool and taxon group concepts. b) illustrates the status of the priority CWR inventory in Sudan and South Sudan for crop improvement, categorized as potential, confirmed, confirmed and potential, and unconfirmed.

Table 1. Relatedness of CWR taxa and their confirmed use in crop improvement in the priority inventory of Sudan and South Sudan. GP1, primary gene pool; GP2, secondary gene pool; GP3, tertiary gene pool. 'b' indicates the wild or weedy form.

Taxon	**Relatedness to CWR**	**Confirmed use of CWR to broaden crop improvement**	**References**
Coffea canephora var. *gossweileri* A. Chev.	GP2b	Coffee berry disease resistance; coffee rust resistance; root-knot nematode resistance	Anthony *et al* (2011); Levi *et al* (2005); Noir *et al* (2003); Prescott-Allen and Prescott-Allen (1988)
Coffea liberica Hiern	GP2b	Coffee rust resistance	Anthony *et al* (2011); Prakash *et al* (2009); Prescott-Allen and Prescott-Allen (1988)
Fragaria chiloensis (L.) Duchesne.	GP1b	Fruit size; Fruit quality	Ahmadi and Bringhurst (1992)
Fragaria vesca L.	GP3	Anthracnose resistance; Powdery mildew resistance; improved aroma	Ahmadi and Bringhurst (1992); Scott (1951)
Fragaria virginiana Duchesne.	GP1b	Fruit number; fruit size; powdery mildew resistance; scorch resistance; day neutral	Ahmadi and Bringhurst (1992); Hancock *et al* (2002)
Diplotaxis erucoides (L.) DC.	GP3	Alternaria blight resistance; blackleg resistance; cytoplasmic male sterility	Klewer *et al* (2003); Prakash *et al* (2009); Siemens (2002)
Diplotaxis harra (Forssk.) Boiss.	GP3	Gene transfer	Begum *et al* (1995)
Eleusine africana K. OByrne	GP1b	Fertility trait	Dida and Devos (2006)
Eleusine kigeziensis S.M.	GP1b	Fertility trait	Dida and Devos (2006)
Thinopyrum junceum (L.) Á. Löve	GP3	Soil salinity tolerance	Nevo and Chen (2010)
Gossypium longicaly x Hutch. & B.J.S. Lee.	GP2	Reniform nematode resistance	Robinson *et al* (2007)
Ipomoea purpurea (L.) Roth.	GP3	Gene transfer	Cao *et al* (2009)
Lens ervoides (Brign.) Grande	GP2	Seed size; Yield improvement; anthracnose resistance; *Ascochyta* blight resistance; *Stemphylium* blight resistance	Ahmad *et al* (1997); Kumar *et al* (2014); Tullu *et al* (2011)
Lupinus mexicanus Cerv. er Lag.	GP3	Gene transfer	Busmann-Loock *et al* (1992); Clements *et al* (2005)
Malus sylvestris Miller	GP1b	Agronomic trait	Volk *et al* (2015)
Manihot carthagenensis subsp. *glaziovii* (Müll. Arg.) Allem	GP2	Cassava bacterial blight; resistance; cassava mealy bug resistance; Cassava mosaic Virus Resistance	Hahn *et al* (1980); Hajjar and Hodgkin (2007); Nair and Unnikrishnan (2007); Prescott-Allen and Prescott-Allen (1988)
Medicago arborea L.	GP3	Anthracnose resistance	Armour *et al* (2008); Quiros and Bauchan (1988)
Olea europaea subsp. *cuspidata* (Wall. ex G. Don) Cif.	GP2	Crop ontology trait	Hannachi *et al* (2009)

Continued on next page

Table 1 continued

Taxon	Relatedness to CWR	Confirmed use of CWR to broaden crop improvement	References
Oryza brachyantha A. Chev. & Roehr.	GP2	Bacterial blight resistance	Brar and Singh (2011)
Oryza longisteminata A. Chev. & Roehr.	GP1b	Drought tolerance; yield improvement; bacterial blight resistance; Grassy stunt resistance	Brar and Singh (2011); Hajjar and Hodgkin (2007); Jena (2010)
Pistacia khinjuk Stocks.	GP2	Rootstock	Hormaza and Wünsch (2007)
Pennisetum purpureum Schumach.	GP2	Cytoplasmic male sterility; fertility restoration genes; panicle length; days to maturity; yield improvement	Dujardin and Hanna (1989) Hajjar and Hodgkin (2007); Hanna (1997); Palit *et al* (2014)
Pennisetum squamulatum Fresen.	GP2	Fertility restoration genes	Dujardin and Hanna (1989)
Phaseolus coccineus L.	GP2	Aluminium tolerance; yield improvement; angular leaf spot resistance; anthracnose resistance; bean stem maggot resistance; bean yellow mosaic virus resistance; common bacterial blight resistance; fusarium root rot resistance; white mould resistance	De Ron *et al* (2015); Freytag *et al* (1982); Loskutov and Rines (2011); Mahuku *et al* (2003); Miklas *et al* (1999); Porch *et al* (2013); Schwartz and Singh (2013); Singh *et al* (2008); Singh (2001); Wilkinson and Re (1983); Zapata *et al* (2004)
Saccharum spontaneum L.	GP2	Cold tolerance; red rot resistance; smut resistance; sugarcane mosaic virus; early maturing	Cordeiro *et al* (2003); Prescott-Allen and Prescott-Allen (1986)
Setaria viridis (L.) P. Beauv.	GP1b	Triazine resistance	Darmency and Pernes (1985)
Sinapis arvensis L.	GP2	Blackleg resistance; sclerotinia resistance; cytoplasmic male sterility	Hu *et al* (2002); Snowdon *et al* (2000); Wei *et al* (2010)
Solanum aethiopicum solan L.	GP3	Rootstock; yield improvement; bacterial wilt resistance; fusarium wilt resistance	Collonnier *et al* (2001); Daunay (2008); Frary *et al* (2007); Rotino *et al* (2014); USDA (2011)
Solanum incanum L.	GP2	Drought tolerance; rootstock; verticillium wilt resistance	Frary *et al* (2007); Knapp *et al* (2013); USDA (2011)
Solanum linnaeanum Hopper & Jaeger	GP2	Fungal wilt resistance	Frary *et al* (2007); Rotino *et al* (2014); Yin *et al* (2015)
Solanum macrocarpon L.	GP3	Rootstock	USDA (2011)
Solanum marginatum L. f.	GP3	Gene transfer	Borgato *et al* (2007)
Sorghum purpureosericeum (Hochst. ex A. Rich.) Schweinf. & Asch.	GP3	Sorghum shoot fly resistance	Nwanze *et al* (1990)
Vigna radiata var. *sublobata* (Roxb.) Verdc.	GP1b	Bruchid resistance	Konarev *et al* (2002)
Vigna vexillata (L.) A. Rich	GP3	Gene transfer	Gomathinayagam *et al* (1998)

The development of the national checklist and inventory of CWR focused exclusively on those associated with food crops, such as rice, sorghum and finger millet. This focus is justified by the crucial role these crops play in providing nutrition and ensuring food security in these two nations.

This region has historically been affected by food insecurity as a direct consequence of social conflict and warfare, making the prioritization of food security crops in the CWR inventory a strategic approach.

Climate change poses a significant threat to the future of food crops, including their wild relatives. Jarvis *et al* (2008) emphasized the critical need to identify and conserve CWR that are threatened by climate change, such as cowpea (*Vigna*), a crucial food security crop in sub-Saharan Africa. Studies by Jarvis *et al* (2008) estimate that 2-6% of *Vigna* species in sub-Saharan Africa could face extinction by 2055, highlighting the urgency of identifying and conserving these threatened CWR. Fortunately, Sudan and South Sudan contain three vital CWR of *V. unguiculata* subsp. *dekindtiana* (Harms) Verdc, *V. unguiculata* subsp. *pubescens* (R Wilczek) pasquet and *V. vexillata* (L.) A. Rich. These CWR represent a valuable genetic reservoir that could be important for developing climate-resilient cowpea varieties in the future, potentially preserving food security in the region and beyond.

CWR conservation priorities are an important step in conservation planning at the national, regional and international levels. This newly developed inventory in Sudan and South Sudan identifies CWR associated with essential food crops such as sorghum, rice, cowpea and pearl millet. While this inventory represents a valuable resource, the number of taxa in Sudan and South Sudan (449 taxa) is lower compared to the checklists of other countries such as Indonesia (Rahman *et al*, 2019), China (Kell *et al*, 2015), Portugal (Magos-Brehm *et al*, 2008), USA (Khoury *et al*, 2013) and Zambia (Ng'uni *et al*, 2019). This difference highlights the importance of continued CWR conservation and exploration efforts in Sudan and South Sudan.

Conclusion

This study highlights the important role of CWR in Sudan and South Sudan in enriching crop diversity and promoting sustainable food production at all levels – national, regional and international. By identifying and prioritizing 85 CWR taxa from a comprehensive checklist of 449, this research provides a crucial foundation for targeted conservation efforts. The establishment of the first CWR checklist and inventory for these two countries offers the basis for further research to ensure the long-term sustainability and utilization of the prioritized CWR. Key areas for future research include:

- Protecting genebanks during civil conflicts: The ongoing civil conflict in Sudan tragically exemplifies this threat. Researchers were forced to call upon the international community to intervene and protect the country's main seedbank from the potential loss of irreplaceable crop varieties and damage to its facilities (Nordling, 2024). Similar situations have been observed with ICARDA in Syria (Darvish *et al*, 2023) and Yemen (Aljarmouzi *et al*, 2024) This incident highlights the urgent need for a comprehensive regional and global initiative to safeguard genebank during conflicts.
- Enhanced floras and CWR inventory validation: Develop separate, comprehensive floras for Sudan and South Sudan, collaborating with agronomists to validate the CWR inventory accuracy.
- Taxonomic expeditions for new CWR discovery: Conduct taxonomic research projects in remote, untapped areas, potentially leading to the discovery of new CWR, and work with national genebanks on collaborative efforts and germplasm preservation.
- Gap analyses: Initiate *in situ* and *ex situ* conservation gap analysis for the priority CWR taxa in each country. These analyses will inform the development of comprehensive conservation plans for each CWR's specific needs.
- Climate change impact assessment: Assessing climate change models to evaluate potential threats to CWR populations.

Supplemental data

Supplemental Table 1. Checklist of CWR in Sudan and South Sudan

Supplemental Table 2. Priority inventory of CWR in Sudan and South Sudan

Supplemental Table 3. Related crop and concept level of the priority inventory of CWR taxa in Sudan and South Sudan

Author contributions

Ahmed Aldow: Conceptualization, data curation, formal analysis, investigation, methodology, resources, validation, visualization, writing (original draft, review and editing). Joana Magos Brehm: Supervision. Maha Kordofani: Resources, validation. Fatouma Abdoul-latif: Resources, validation. Nigel Maxted: Supervision.

Conflict of interest statement

The authors confirmed that no conflict of interest exists.

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Genetic improvement of indigenous cattle breeds in Ethiopia: A systematic review of the Fogera cattle open nucleus breeding scheme

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Abstract: Fogera cattle are one of the valuable indigenous milk-type local breeds of Ethiopia, widely adapted to the area around Lake Tana in the Amhara region. The objective of this systematic review was to evaluate the performance of the Fogera cattle breed under an open nucleus breeding scheme. The review was done systematically by collecting published and unpublished data sources on the breed. The overall milk yield of the nucleus Fogera cattle herd was 2.26±0.794L/day. From the total herd, the top 10% and 25% of them produced daily milk yields of 3.31 and 2.87L, respectively, and some elite cows gave an average of 5.45±0.73L/day with a maximum yield of 8L/day. The predicted 305-day milk yield for the top 10% and 25% of the total herd was 883.64 and 772.83L, respectively. The average lactation milk yield and lactation length were reported to be 489±184L and 243±72.79 days, respectively. The respective heritability estimates for the aforementioned traits were 0.20±0.23 and 0.27±0.001. The birth and weaning weights (at 8 months of age) of village Fogera cattle born from community-based breeding programmes (CBBP) were 23.77±.21 and 85.89±1.07kg, respectively. The average weaning age for the CBBP herds was reduced to 8 months. The overall calf mortality in the nucleus herd was 3%. The CBBP demonstrated that it could act as a significant entry point for ensuring the conservation and restocking efforts of this breed as a country asset.

Keywords: Community-based breeding programme, Ethiopia, Growth traits, Milk yield, ONBS, Reproductive traits

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Introduction

Indigenous animal genetic resources are believed to preserve much of the current global genetic diversity, with millions of people directly depending on them (FAO, 2023). Indigenous cattle breeds constitute an important reservoir of genetic material, for which developing nations have failed to provide adequate recognition through their sustainable use and conservation, which puts them at risk of extinction. For example, Sheko cattle in Ethiopia are highly threatened because of crossbreeding with other local cattle, and others like Fogera, Begayit, Ogaden and Borena cattle breeds in Ethiopia are also facing various degrees of threat that challenge their existence as a breed (IBC, 2004). These breeds are decreasing and deteriorating in terms of both population size and genetic diversity due to paradigm shifts in the existing farming system and production system and farm size dynamics of the native habitat, leading to a subsequent genetic dilution (Kebede *et al*, 2013; Tesfa *et al*, 2017; Adisu *et al*, 2021).

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The increasing demand for dairy products because of high population growth, urbanization and improved standards of living is pushing Ethiopia's livestock keepers to increase the productivity of their animals (Shapiro *et al*, 2015). Shapiro *et al* (2017) indicated that, to realize genetic improvement programmes in Ethiopia, the best contributors are a range of agro-climates and production systems, feed and water availability, and small to large-scale farms. Since the 1960s, selective breeding and crossbreeding have been implemented for the genetic improvement of Ethiopian cattle (Chebo and Alemayehu, 2012). However, the anticipated improvement in milk production and productivity has not yet been achieved, even with crossbreeding. The reasons for this, as indicated by Shapiro *et al* (2015), include separate implementation interests between research and development officials, the absence of a clear and implementable strategy that targets the improvement scenario of indigenous breeds, and the absence of sustained support from different stakeholders.

Fogera cattle are one of the promising breeds of Ethiopia that are widely adapted to the plain of Lake Tana in the Amhara region. The Fogera cattle breed is known for its relatively higher milk yield, larger body frame and traction power, better resistance to internal parasite infestation, and sound adaptability to waterlogged Fogera plains, which is attributed to its long legs (Tesfa, 2015). The milk production potential and draught power of the breed are farmers' preferred traits (Zewdu, 2004; Bitew *et al*, 2007). In its production environment, the breed is used as a dam line for milk yield improvement (with Holstein Frisian semen) while the bulls are used in crossbreeding with local highland zebu cattle, which perform poorly under smallholder production system, to improve milk yield, growth rate and draught power (Tesfa *et al*, 2017, 2022). The population size of Fogera cattle is declining at an alarming rate, therefore urgently requiring conservation efforts for future potential use of the breed.

The Andassa Livestock Research Center (ALRC) has been implementing selective breeding and conservation efforts both on station and on farm to safeguard the Fogera breed from extinction and increase its productivity. The center was established in 1964 as the Imperial Fogera Cattle Conservation Centre, with 57 Fogera cows and three bulls purchased from the local area. It was then re-organized as a farm in 1980-1981 and started its operation with the main objective of conserving Fogera cattle and producing F1 Holstein × Fogera crossbreds for distribution to farmers to increase milk production. It was upgraded into a livestock research centre in 2000 (ALRC, 2017). However, in its 40-year journey, it did not provide any visible, significant and sustainable positive change to farmers' lives. Among the reasons is the inability to design and formulate an effective breeding programme. This is a key problem not only for ALRC case but also for all farms, ranches and multiplication centres found in the country.

In 2007, an open nucleus breeding scheme linking the ALRC nucleus and the village herds was designed for Fogera cattle improvement, and the programme was also used as a model for other breeds under different research centres, farms and multiplication centres. The ultimate objective of the breeding scheme was to restock the declining village Fogera cattle populations and improve the livelihood of the farmers. This manuscript summarizes the productive performances, past achievements, shortcomings and lessons learned under the open nucleus breeding scheme and community-based breed programme (CBBP) and indicates gaps and future directions for the sustainability of breeding initiatives.

Material and methods

Data sources

Various researchers, professionals and students (PhD and MSc) involved in the Fogera cattle breeding programme published articles, proceedings and case studies at the level of the two nucleus herds (ALRC and CCBIR) as well as on the community herds. From the summary, above 10 MScs, 2 PhD dissertations, 42 published journal articles and above eigh national, regional, and societal proceedings had information regarding Fogera cattle production and productivity. Besides, more than ten articles have used the genetic potential of Fogera cattle in comparison with other national and internationally recognized breeds for genetic diversity, selection signatures and molecular conservation studies. For this case study, data from more than 17 papers and thesis works with full information on the breed done under the open nucleus breeding scheme were analyzed and compared, while research articles with little information on the Fogera cattle were used to discuss and supplement the main findings. The collected data were analyzed by SAS (2002) and MS Excel was used to develop graphs and trend lines. Besides, articles published online on different Ethiopian breeds were used to discuss the main findings of the case study (Table 1).

Data analysis

Daily milk yield (L), lactation milk yield (L), birth weight (kg) and weaning weight (kg) performance were the dependent variables, while breeding period (year), sex, season and district were used as independent variables for the analysis of quantitative traits. The fixed factors were analyzed by the GLM procedure of SAS (2002) software. Genetic parameter estimation for pre-weaning growth performance and milk-related traits was done by the statistical procedure of Wombat software (Meyer, 2007) in the case of Kassahun *et al* (2020) and ASReml (Gilmour *et al*, 2015) in the case of Tesfa (2015). Heritability, repeatability, and genetic and phenotypic correlations were estimated. Survival of calves at the nucleus herd was done with the Cox proportional hazards model (Cox, 1972) of Stata software (SE 14) (1996–2021) in the case of Gessesse

Table 1. Data sources used for the systematic review. ALRC, Andassa Livestock Research Center; CCBIR, Chagni Cattle Breeding and Improvement Ranch.

Publication types	Publication/ releasing years	Articles' focus	Working sites	Study topics	No. of publications
MSc thesis	1992 to 2019	Fogera cattle breed	ALRC, CCBIR and on farm	Growth, milk, reproduction, survival	6
Scientific articles	2005 to 2023	Fogera cattle breed	ALRC, CCBIR and on farm	Growth, milk, reproduction, survival, population number	15
	1982 to 2023	Ethiopian indigenous cattle breeds	Different sites in Ethiopia	Production system, growth, milk, reproduction	10
Proceedings	2007 to 2019	Fogera cattle breed	ALRC, CCBIR and on farm	Production system, growth, milk, reproduction, survival	5
Reports and working documents	2004 to 2023	Fogera and other Ethiopian indigenous cattle breeds	Different sites in Ethiopia	Production system, breeding design, strategic plan	8

et al (2021a), and the chi-square test of SPSS (version 22) was used in the case of Mola *et al* (2019).

Results

Description, distribution and adaptive potential of Fogera cattle

The Fogera breed is characterized and well known by its pied coat of black-and-white or black-and-grey; short, stumpy, pointed horns; hump ranging from thoracic to cervico-thoracic; folded dewlap, of moderate to large size; docile temperament; and is used for draught, milk and meat (Rege and Tawah, 1999; DAGRIS, 2007). It is highly tolerant or resistant to heat stress and solar radiation, which could be due to its dominantly white coat colour with short hair. Additionally, the breed is known for its adaptation to high altitudes, tolerance to parasite and disease infestation, fly burden, wet soils or swampy areas, low-quality feed and other unfavourable environmental conditions (Alberro and Haile-Mariam, 1982).

Farmers keeping Fogera cattle reported the breed is known for its drought tolerance, better milk yield and growth rate (Tesfa *et al*, 2022), and its environmental adaptation and meat production potential (Kassahun, 2019). The breed is reared in districts surrounding Lake Tana and is one of the most populous and productive breeds in the Amhara region (Tesfa, 2015) and the country (IBC, 2004). Additionally, the breed is found and conserved at ALRC, Chagni Cattle Breeding and Improvement Ranch (CCBIR), and their surrounding kebeles (a small administrative unit in Ethiopia). Figure 1 shows the Fogera cattle distribution districts and working sites.

Description of the breeding strategy

The open nucleus breeding scheme (Figure 2) was the strategy employed since 2007 to conserve, improve and restock the declining population of Fogera cattle. The nucleus was established in early 1964 by the Emperor's regime (ALRC, 2017). The scheme was implemented in selected districts where the pure line breed was intended to be produced, namely Wagetera kebele of Fogera District and Metrha Abawarka kebele of Gondar Zuria District. However, due to high levels of admixture observed in the village herd, the breeding scheme has been closed since 2015. This has prevented the introduction of heifers from the village herd to the nucleus herd to safeguard the latter from genetic dilution (Figure 3; Tesfa *et al* (2017)).

The districts involved in the open nucleus breeding scheme study were selected through the participation of researchers and experts, with criteria such as the presence of true-to-type Fogera cattle (50%), accessibility and presence of knowledgeable farmers (25%), and others like the willingness of farmers, availability of communal grazing land, and enough land for feed development (25%). After the selection, a discussion was done with the community on points like the importance and productivity of the breed, its value for them, and the need for conservation and improvement strategies. After a consensus was built with the community, farmers were selected to hold the breeding bulls and serve the community. Those farmers were selected based on wealth status, cattle management practices and the presence of a better educational background for record keeping. The bulls were provided under a written contractual agreement for four years of service to avoid inbreeding, after which the bulls became the property of the recipients. The participating farmers were then arranged as a community-based breeding programme, having their own committee to manage and decide on the activity with the researchers leading the programme (Tesfa *et al*, 2019).

The meta-analysis was done for production traits like daily milk yield, lactation milk yield, lactation length;

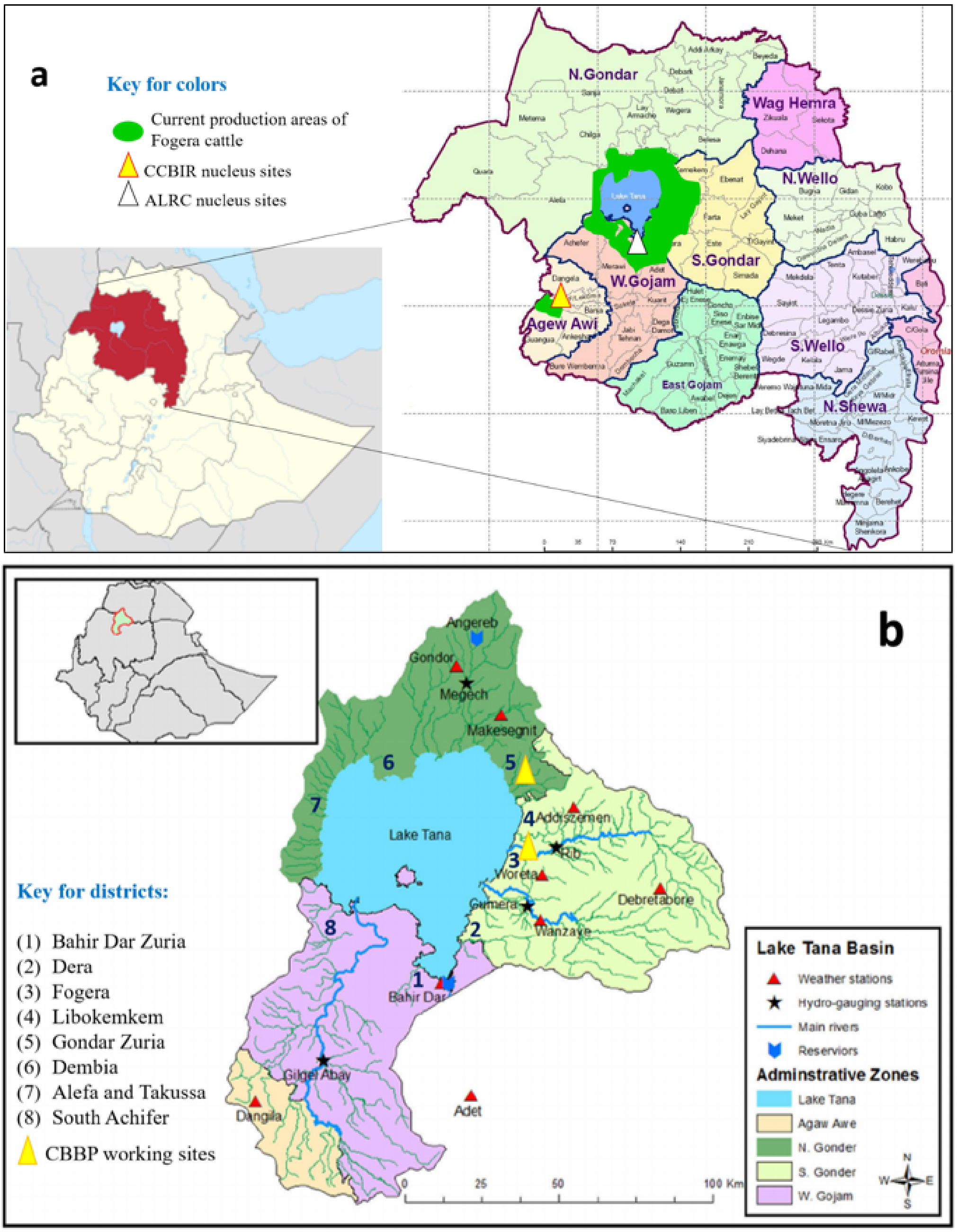

Figure 1. a) Distribution of Fogera cattle in the Amhara region, and Andassa Livestock Research Center (ALRC) and Chagni Cattle Breeding and Improvement Ranch (CCBIR) nucleus sites (Tesfa *et al*, 2022); b) Districts suitable for the production of Fogera cattle breed and community-based breeding programmes (CBBP) working sites (Tesfa *et al*, 2017)

growth-related traits for birth weight and weaning weight; reproductive performance traits for age at first calving, calving interval, and days open; and calf survival in Fogera cattle kept under an open nucleus breeding scheme.

Productive and reproductive performances

Milk yield performance of Fogera cattle

According to the report of Tesfa *et al* (2019), the milk yield of Fogera cattle at ALRC showed an increasing trend from 2002 to 2017, with a lower yield between 2005 and 2007. The inbreeding rate during this time, according to the author, contributed to this outcome. Removing the inbred individuals increased the output from 1.92 to 2.43L per day. From the total herd, the top 10% and 25% had an average milk yield of 3.31L and 2.87L per day, respectively. At ALRC, there were four groups of pure nucleus herds with an average of 45 cows and one mating bull. These were selected and grouped based on their estimated breeding value (EBV) for milk yield and family relationships to minimize inbreeding; the top 45 individuals with higher EBV were grouped as group I, and next 45 individuals with group II and the like for group III and IV (Table 2).

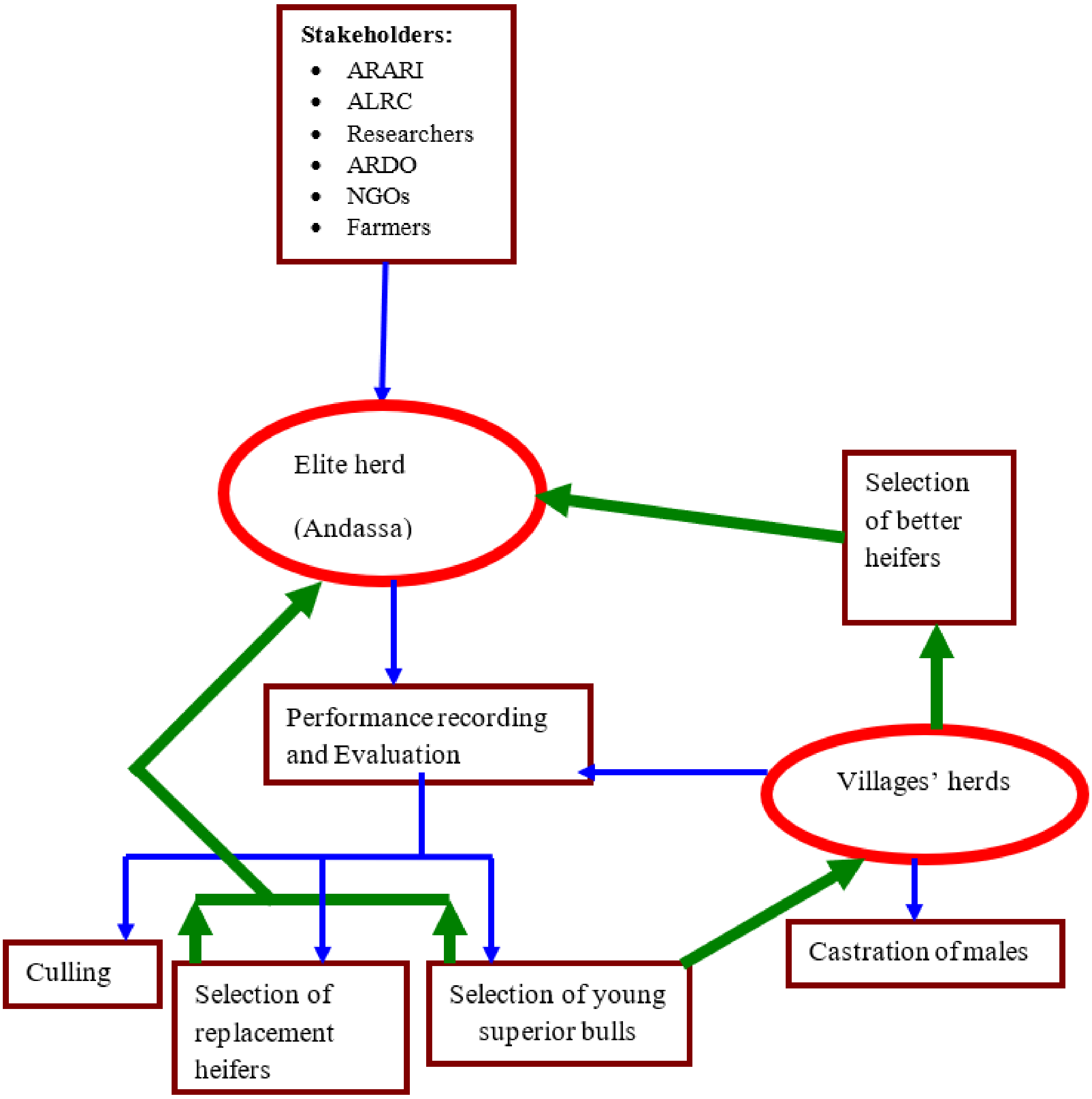

Figure 2. Open nucleus breeding scheme (ONBS) (Tesfa *et al*, 2019)

As indicated in Table 2, the daily milk yield per cow had a big variation from 0.4 to 7.2L per day, which still allows for improvement within breed selection. The predicted 305-day milk yield also indicated a big variation among breeds and ranged from 274 to 1,194L of milk with an overall average of 578.26L. The top 10% and 25% of the total herd had a 305-day milk yield of 883.64 and 772.83L, respectively (Tesfa *et al*, 2019). According to the experiment by Bitew *et al* (2021) on the potential exploitation of the breed for milk yield, cows were divided into two groups: group one received concentrate feed adjusted based on their daily milk yield in addition to grazing, while group two relied on grazing alone (control). The cows in group one gave 4.69L of milk per day compared to 2.21L in the control group. This indicated that the breed's genetic potential may have been masked by poor environmental conditions, which need to be improved through delivering additional feeds and husbandry practices.

According to Kassahun *et al* (2020), the average daily milk yield (DMY) and lactation length (LL) of the breed were 1.98±0.60L and 243±72.79 days, respectively, and the lactation milk yield (LMY), which is calculated by multiplying DMY by LL, was 489±184L. As shown in the trend (Figure 4), the results for these parameters exhibited significant variability and inconsistency over the reported years, which was due to the lower selection intensity and number of animals considered in the selection procedure (Kassahun *et al*, 2020). According to the genetic parameters estimate, heritability for DMY, LL and LMY was 0.33±0.27, 0.20±0.23, and 0.27±0.001, respectively. Besides, the repeatability of the respective traits of interest was 0.33, 0.48, and 0.55 (Kassahun *et al*, 2020).

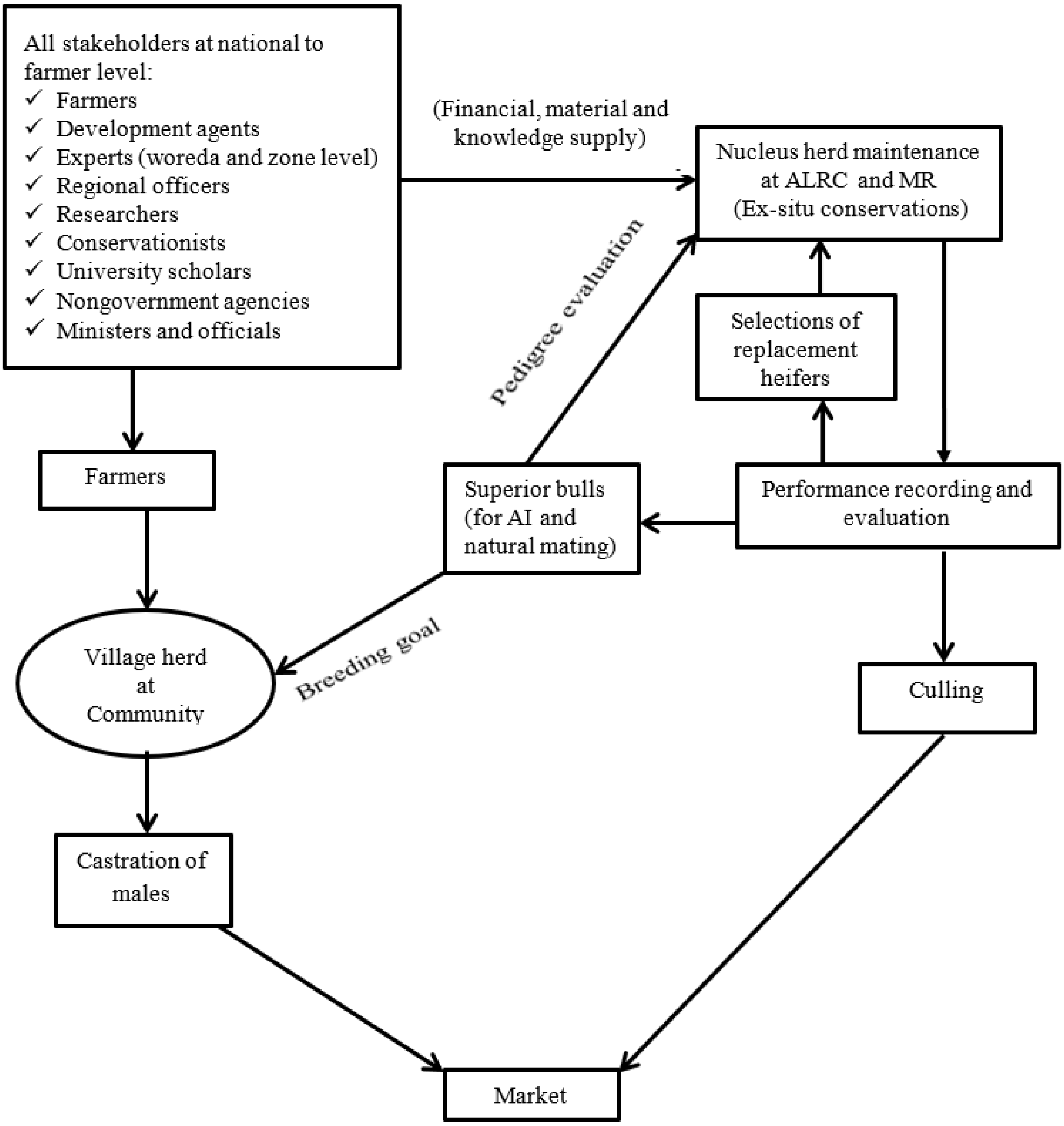

Figure 3. Closed breeding scheme (Tesfa *et al*, 2017)

Table 2. Milk yield performance of the four pure nucleus herds at the Andassa Livestock Research Center (ALRC). Source: Tesfa *et al* (2019)

Herd type	**Milk yield (L/day)**			
	Mean	**SD**	**Minimum**	**Maximum**
Overall	**2.26**	**0.794**	**0.4**	**7.2**
Fogera Group I	2.24	0.731	0.7	5.5
Fogera Group II	2.52	0.863	0.6	6.1
Fogera Group III	2.05	0.814	0.4	7.2
Fogera Group IV	2.18	0.707	0.6	4.5

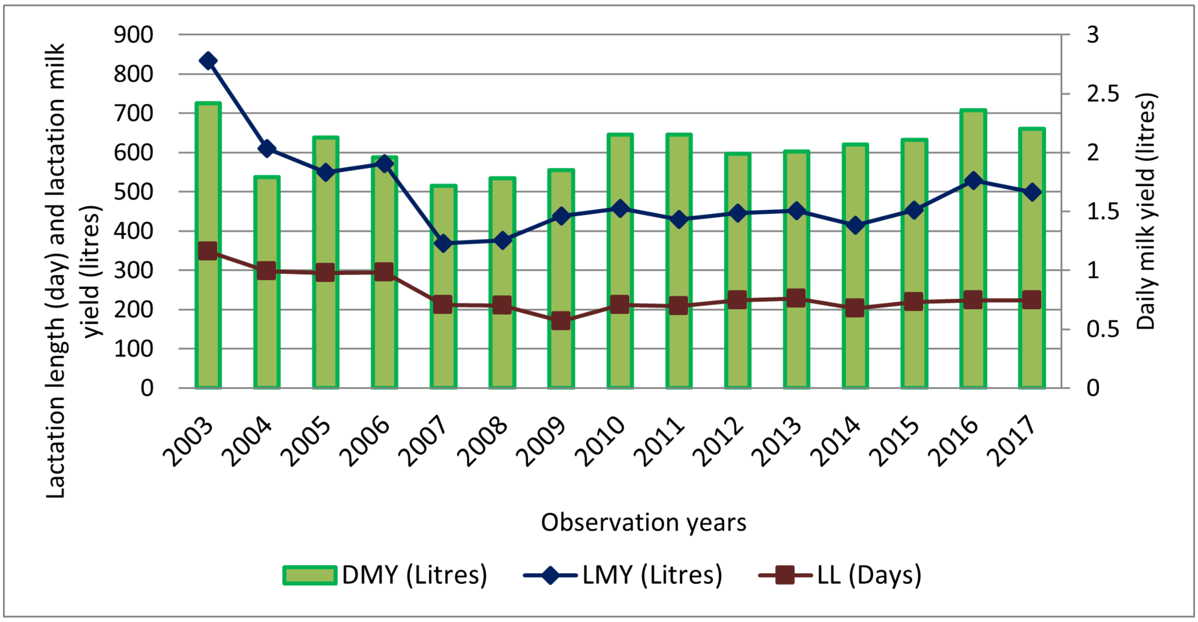

Figure 4. Lactation length (LL), lactation milk yield (LMY) and daily milk yield (DMY) of Fogera cattle across selection years (Kassahun *et al*, 2020)

Growth performances

Performance under Nucleus herd. The birth and weaning weight of Fogera calves in the two nucleus herd populations is presented in Figure 5. According to the trend across selection years, both weaning and birth weight showed variability in both sites. The main reasons for this inconsistency were variations in the management of pregnant cows and calves, climate variability exerting an effect on feed availability, poor recording systems, and differences among individual animals included in the selection process during different years (Bekele, 2012; Tesfa *et al*, 2016; Kassahun *et al*, 2022). Besides, the observed inbreeding in the nucleus herd during 2006-07 (Mekuriaw and Bitew, 2006) and varying management aspects (Kassahun *et al*, 2022) at ALRC contributed to the declining trend of birth and weaning weight of Fogera calves.

The heritability estimate for the pre-weaning growth rate of Fogera cattle at the two nucleus herds is summarized in Table 3. It was observed that the heritability for birth weight had declined with the advancement of years at ALRC. Differences across years might be due to management differences that influence the environmental part of the estimated genetic parameter, the number of data points, and differences in methods of estimation among the authors. The heritability estimate at CCBIR was lower compared to ALRC; even the estimate for the F1 crossbreed between the Fogera and Holstein Friesian was lower for birth weight and pre-weaning average daily gain (PWADG) and comparable for weaning weight (Table 3). The lower estimate at CCBIR for pre-weaning growth parameters was attributed to the presence of management variations, poor nutritional status of the animals, high environmental stress and data record quality (Bekele, 2012; Zeleke, 2014).

Performances under community-based breeding programme (CBBP) . As an open nucleus breeding scheme, ALRC distributed about 17 pure improved Fogera bulls selected based on their estimated breeding value and physical soundness in two kebeles to conduct a community-based breeding programme since 2012. Trained enumerators collected data for 8 conscutive years on birth and weaning weight from 2,180 calves born by distributed improved bulls and village local bulls for performance comparison. The birth and weaning weights (at 8 months of age) of the village herds born from improved bulls were 23.77±0.21 and 85.89±1.07kg, respectively (Tesfa *et al*, 2019). The average weaning age was lowered from one year to 8 months. Based on the monitoring data at CBBP villages, the average birth and weaning weight of calves born from village local bulls were 20.21 and 85.14kg (at 1 year of age), respectively. This indicated that attempts made through the open nucleus breeding programme, beyond the conservation and restocking efforts, can achieve the genetic improvement of the indigenous Fogera cattle breed (Tesfa *et al*, 2019).

Strategic deworming of internal parasites and scheduled vaccination against known diseases (anthrax, lumpy skin disease, foot and mouth disease and bovine pasteurellosis) were implemented as part of the flock health monitoring. Additionally, grazing land management, the introduction of waterlogged tolerant grass varieties and fattening technology for castrated village local bulls were introduced to CBBP villages to improve the production environment and create an income source for producers. According to (Tesfa *et al*, 2019), under the CBBP, the age at first calving (AFC) was reported to be 36 months, and compared with the

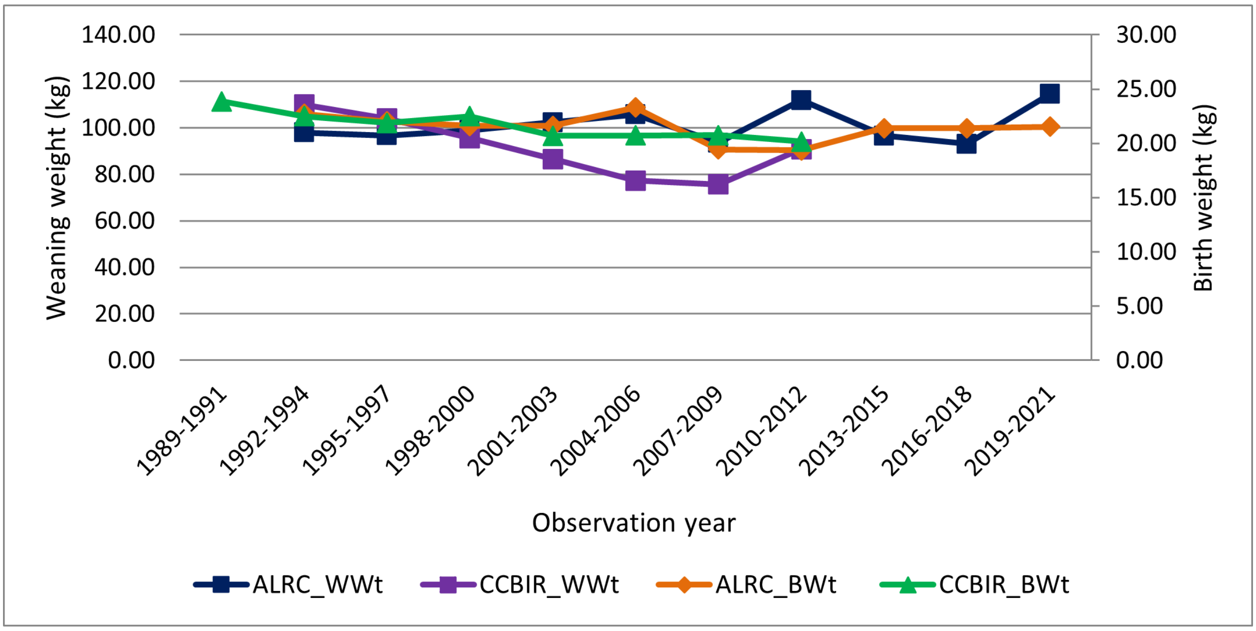

Figure 5. Birth weight and weaning weight at the two nucleus herds. ALRC_BWt, birth weight at the Andassa Livestock Research Center (ALRC); CCBIR_BWt, birth weight at the Chagni Cattle Breeding and Improvement Ranch (CCBIR); ALRC_WWt, weaning weight at ALRC; CCBIR_WWt, adjusted weaning weight at CCBIR (Bekele, 2012; Tesfa *et al*, 2016; Kassahun *et al*, 2022).

Table 3. Direct heritability estimate for pre-weaning growth traits at the two nucleus herds. BWt, birth weight; WWt, weaning weight; PWAGD, pre-weaning average daily gain; *, estimates were done for Fogera × Holstein Friesian (F1).

Parameters	BWt	WWt	PWADG	References
ALRC	0.38±0.32	0.22±0.25		Sewalem (1992)
	0.24±0.09	0.18±0.05		Tesfa *et al* (2019)
	0.21±0.07	0.26±0.01	0.55±0.19	Kassahun *et al* (2022)
CCBIR	0.03±0.02	0.06±0.03	0.05±0.03	Bekele (2012)
	0.13±0.04	0.24±0.08	0.16±0.07	Zeleke (2014)*

actual results before the programme, there was a one-year shortening of AFC. This shows how pure Fogera bulls contributed to upgrading the genetic makeup of the village herds. As traction power is a selective trait of the farmers, calves born through the breeding programme started ploughing at 31 months of age, while the farmers' local bulls did the same work at 41 months Tesfa *et al* (2019). The positive contribution of the open nucleus breeding programme at the on-farm level to the improvement of the genetics and environment was appreciated by participant farmers (Kassahun, 2019).

Reproductive performances of nucleus herd

The average reproductive performance of Fogera cattle at ALRC and CCBIR is presented in Figure 6. The overall average age at first calving (AFC) of 52.00±3.27 months and 52.17±3.17 months; calving interval (CI) of 19.86±2.15 months and 18.65±1.12 months; and days open (DO) of 341.62±90.89 and 280.27±53.86 days were reported at ALRC and CCBIR nucleus herds, respectively. The AFC (month) and DO (day) were lower at ALRC nucleus herds compared with CCBIR's while CI (month) was shorter in the CCBIR nucleus herd compared with the ALRC's (Figure 6). Variations between these two nucleus breeding herds might be attributed to the presence of agroecological differences among the sites. Due to the objectives of the breeding scheme, the improvement in reproductive performance was attained through indirect selection with daily and lactation milk yield. A comparable result for DO (9.5 months), higher CI (19.56 months), and lower AFC (50.8 months) was reported for the nucleus herd at CCBIR (Melaku *et al*, 2011) and a slightly lowered DO (10.17 months) (Gebeyehu *et al*, 2005), AFC (51.76 months), and CI (19.53 months) was reported for nucleus herd at ALRC (Tesfa *et al*, 2016). As reported by Sendeku *et al* (2016), the AFC and CI for on-farm Fogera cattle was 51.4±0.05 and 21.18±0.70 months, respectively.

Calf mortality rate

The overall calf mortality at ALRC and CCBIR was 3% (Gessesse *et al*, 2021b), which is comparable with the minimum standard (3–5%) set for the calf mortality rate (Heinrichs and Radostits, 2001). Gessesse *et al* (2021a) reported that season, breed and birth weight had a significant ($P < 0.05$) association with the incidence of calf mortality in both Fogera cattle nucleus herds, with a respective hazard ratio (HR) of

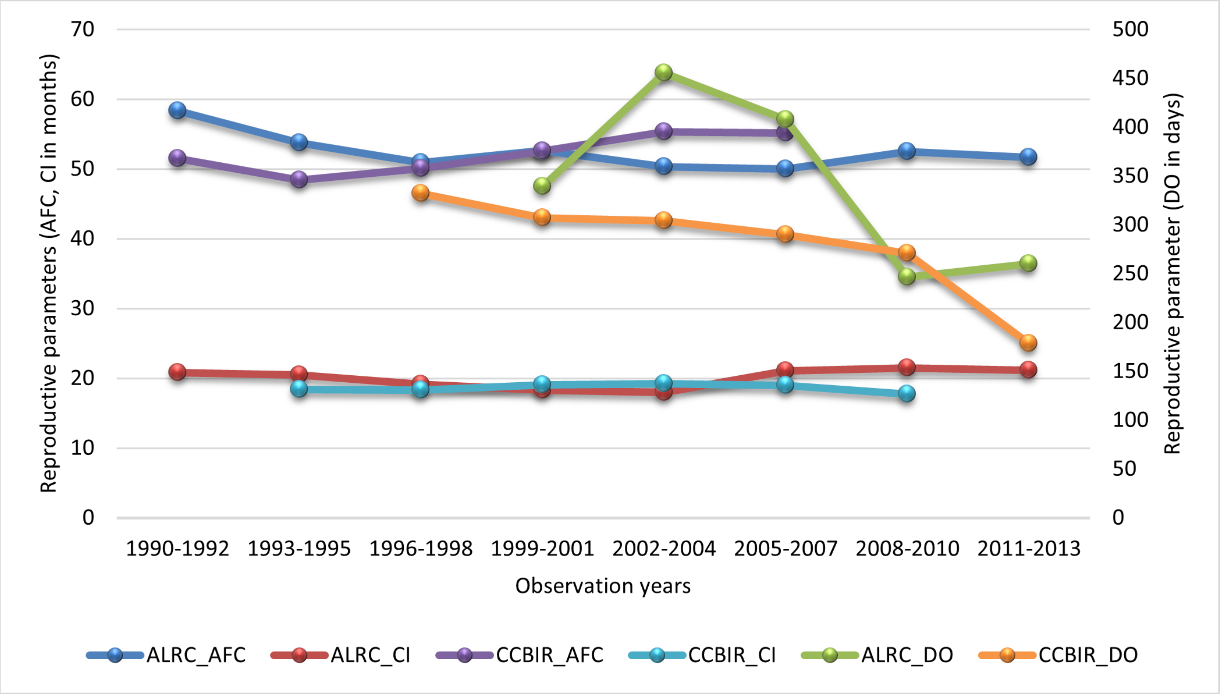

Figure 6. Reproductive performance of Fogera cattle at the two nucleus herds. ALRC_AFC, age at first calving at the Andassa Livestock Research Center (ALRC); CCBIR_AFC, age at first calving at the Chagni Cattle Breeding and Improvement Ranch (CCBIR); ALRC_CI, calving interval at ALRC; CCBIR_CI, calving interval at CCBIR; ALRC_DO, days open at ALRC; CCBIR_DO, days open at CCBIR (Bekele, 2012; Tesfa, 2015; Kassahun, 2019)

1.6, 0.55, and 0.88. Conversely, other potential risk factors such as calf sex, dam parity, year of birth and location did not show a significant effect on calf mortality rates at an early age of the calves at both ALRC and CCBIR (Gessesse *et al*, 2021b). The author indicated that among the significant risk factors, birth weight (HR = 0.88, P = 0.000) was found to be a very important determinant of calf mortality. Based on the health monitoring data, the overall morbidity and mortality rate reported for Fogera calves at ALRC were 12.96% and 7.5%, respectively (Figure 7) (Mola *et al*, 2019), and the diseases contributing to calf mortality were diarrhea, systemic infection, coccidiosis, and gastrointestinal parasites, in order of importance.

On the other hand, the work done at ALRC (Kassahun *et al*, 2023) indicated that the pre-weaning mortality rate of male calves (21.3%) was higher than that of female calves (13.4%). Based on the factors considered, the dry season and lightweight calves contributed more to calf mortality than the wet season and heavyweight calves, respectively. The main reasons for the contribution of these factors were the variability of feedstuffs across seasons and the ability to resist the new environment with a heavier weight at birth, respectively. The heritability estimates done for calf survival at 1, 4 and 8 months of age were 0.26, 0.22 and 0.38, respectively (Kassahun *et al*, 2023), which is categorized as a medium level of heritability.

Population status of Fogera cattle

The estimated population size of the Fogera cattle breed is declining progressively from about 800,000 in the 1980s (Alberro and Haile-Mariam, 1982) to 55,646 heads in 2017 (Tesfa *et al*, 2022), even though Girma *et al* (2016) argued for the presence of satisfactory genetic diversity in Fogera cattle. According to the breed keepers, the population of the Fogera cattle has decreased (40%), increased (13%), is stable (6%) or is not known (41%) (Tesfa *et al*, 2022), while respondents who replied to Kassahun (2019) indicated the population had increased (51.81%), decreased (31.81%), is constant (4.54%) or is not known (11.84%). The difference between the population trends of the two studies is that the respondents to Kassahun (2019) were participants in the open nucleus breeding scheme done by ALRC. Tesfa *et al* (2022) indicated the households in the production track of the breed had kept relatively pure Fogera (41%), Fogera-zebu mix (35%), and highland zebu (24%). The Fogera breed appeared to be threatened due to changes in the agricultural production system in the area and genetic dilution by interbreeding with other adjacent indigenous cattle breeds and by indiscriminate crossbreeding with exotic dairy cattle breeds. Population viability analysis indicated that the pure Fogera cattle are not viable, and population growth is decreasing due to feed shortages, interbreeding with other indigenous breeds, disease and parasites (Alemayehu *et al*, 2015).

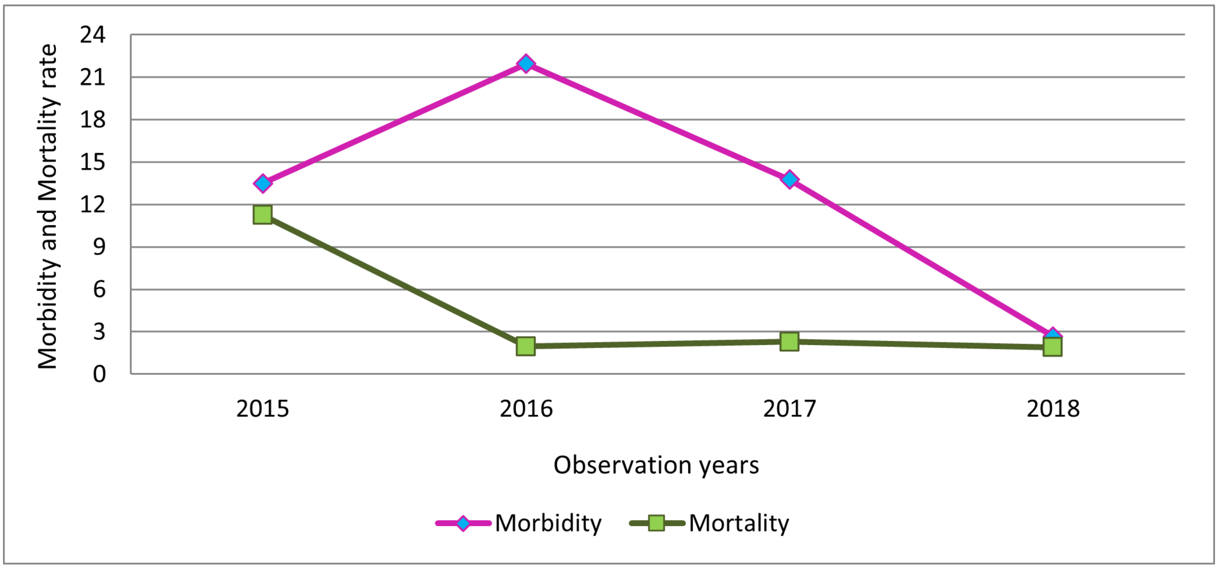

Figure 7. Morbidity and mortality percentage of Fogera cattle at the Andassa Livestock Research Center (ALRC) (adapted from Mola et al., 2019)

Discussion

The daily milk yield of the Fogera cattle at the on-station level indicated a wider variation across selection years, ranging from 0.4 to 7.2L per day, with an average of 2.26L. The reported average daily milk yield (DMY) and lactation milk yield (LMY) were higher than the 1.65±0.03L and 475.85L, respectively, reported for Horro cattle at on-farm conditions (Mekonnen *et al*, 2012) and LMY (425.34±24.06L) at Bako agricultural research centre, Ethiopia (Dabi, 2020). Relatively lower respective values of DMY, LMY and lactation length (LL) of 1.5±0.01L, 419.8±4.45L, and 284.1±0.15 days were reported for Horro cattle at the on-fam level (Mekonnen *et al*, 2021). Similarly, Mekonnen *et al* (2021) reported that the top 10% and 25% of the Horro cattle population produced 2.01 and 1.86L of milk/head/day, respectively, which is lower than that reported for Fogera cattle (Tesfa et al., 2019). Lower average DMY (1.7L) and higher LMY (507L) were also reported for Boran cattle (Haile *et al*, 2011). Mezgebe *et al* (2018) reported a higher average DMY (4.04kg) and average LMY (936kg) for Begait cattle in northern Ethiopia.

The reproductive performance of Fogera cattle observed under both open nucleus and community-based approaches at village herds was comparable with those of other Ethiopian indigenous cattle (Mohammed, 2020; Adisu *et al*, 2021). According to Tenagne *et al* (2023), the performance for calving interval (CI) and age at first calving (AFC) for indigenous cattle in northwest Ethiopia was 20.4±6.1 and 52.5±6.8 months, respectively. A slightly lower result for AFC and CI for Ethiopian Boran cattle at on-station set-up was 48.39±1.41 and 17.91±1.01 months, respectively (Hordofa and Melua, 2021), while a higher value for AFC and CI for the same breed at on-farm level was 53.0 and 18.0 months, respectively (Wario *et al*, 2016). Lower results for AFC (41.2±0.28 months), CI (13.9±0.3 months), and days open (100.5±4.5 days) were reported for Horro cattle in on-station conditions (Jalata *et al*, 2023). The observed variations in the reproductive performance traits between the Ethiopian indigenous cattle breeds might be due to differences in the adaptation of the various local breeds that make them useful for husbandry in different areas and their genetic diversity should therefore be conserved through appropriate programmes.

Conclusion and ways forward

This systematic review summarizes the efforts towards genetic improvement and conservation of Fogera cattle under the open nucleus breeding scheme at ALRC and CCBIR as a nucleus herd and at the on-farm level under CBBP. The results indicate that there was an improvement in milk and growth-related traits, although the trend was slow and decreasing. The presence of variation among individuals in traits of interest suggested there is room for improvement of the breed through selection. One can also assume that the breeding programme was successful, but there are still different problems masking the genetic potential, such as feed availability, management system of dams, data recording, and transfer of data from one researcher to the other. The closed breeding scheme, which the centre currently follows, has been strengthened with the full involvement of farmers, other stakeholders, researchers and experts at different levels. On top of this, the community-based breed productivity improvement programme acts as a big entry point to assure the conservation and restocking efforts of the breed as a country asset, and the programme is better supported with forage, health and extension works to assure sustainability. With these conclusions, the following recommendations are suggested for sustainable breeding programmes in the future:

- Consistent data records are important for an accurate estimation of genetic and non-genetic parameters for better selection. Thus, a standard record-keeping practice should be adopted.
- The researchers working on the community-based breeding programme should estimate the breeding values of distributed bulls from the nucleus and their daughters for milk and growth-related traits in order to ensure the contribution of a real genetic gain from the distributed bulls.
- The breeding objective developed for milk-related traits should be revised to incorporate the potential of the breed for meat-related traits.
- As heritability for calf survival has been categorized as medium, it is advisable to include it in the selection index under the breeding programme.
- It is recommended to develop a reproductive biotechnology unit to speed up the genetic gain and multiply elite high-yielding animals, which should be established at one of the nucleus sites.

Author contributions

Assemu Tesfa and Kefyalew Alemayehu contributed to the study's conception and design. Data collection and meta-anlysis were done by Assemu Tesfa, Mengistie Taye and Demelash Kassahun. Data analysis and writing of the first manuscript draft were performed by Assemu Tesfa. All authors commented on the various versions of the manuscript, and read and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Genetics solutions for improved chicken production in Ghana

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Abstract: The Tropical Poultry Genetics Solutions project intervention in Ghana, a collaboration between the International Livestock Research Institute and the University of Ghana, with support from Ghana's Ministry of Food and Agriculture, seeks to test the adaptability, productivity and farmer preferences of tropically adapted improved dual-purpose chicken genotypes under different production systems. Poultry farmers from two agroecological zones in five of the 16 regions of Ghana were selected to participate in the project. These farmers were provided with selected tropically adapted chicken genotypes, namely Kuroiler and Hubbard, to manage under their production system, and data was collected on bird performance in terms of body weight, egg production, quality and overall acceptability by the farmers. The findings indicate a high farmer preference for, and significantly better ($p \leq 0.05$) growth performance of the introduced chicken genotypes compared to local chickens. For instance, the introduced birds reached an average weight of 2kg within 18 weeks, compared to local chickens which required 30 weeks on average to reach 1.6kg under semi-intensive production management conditions. Additionally, the egg production and carcass attributes of these selected genotypes were found to be comparable to those of the local chickens. Based on these positive results, we recommend the multiplication and widespread adoption of Kuroiler and Hubbard chicken strains among smallholder farmers in Ghana. By doing so, we anticipate improved chicken production, increased income generation, enhanced livelihoods and protein food security in the country.

Keywords: Chicken breeds, Dual-purpose, Hubbard, Kuroiler, Sustainable Development Goals

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Introduction

Poultry production in Africa is mainly based on free-range indigenous chickens (Dessie *et al*, 2011), which are well-valued in terms of their contribution to nutritious diets, household income and sociocultural attributes (Aboe *et al*, 2006; Dessie *et al*, 2011). Indigenous chickens in Africa are adaptive to the environment, survive on low-energy feeds, are often resistant to endemic diseases, and have been characterized to have good potential to be selected for meat and eggs (Ajayi, 2010; Dessie *et al*, 2011). Thus, African chicken resources constitute a reservoir of useful genes important for adaptation and meeting local breeding goals (Osei-Amponsah *et al*, 2013). Such indigenous chickens are mostly maintained under traditional village production systems, typically characterized by low input and low egg and meat production (Dessie *et al*, 2011; Birhanu *et al*, 2023). In Ghana, smallholder chicken production systems are predominantly based on unimproved genotypes maintained mostly in low-input extensive systems. Despite their unique adaptive attributes, contribution to protein food security, low capital requirements and minimal production risks, their overall productivity remains relatively low. There is a need to select

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and improve indigenous chickens to take full advantage of their adaptive potential and genetic variability (Osei-Amponsah *et al*, 2013). Genetic selection within the indigenous breed ideally relies on accurate data recording, including pedigree, and for the birds to be raised in a similar environment where the selection programmes have occurred. Additionally, a large population with a substantial number of active and reserve sire and dam lines is needed to allow exhaustive exploitation of genetic variations for the meat yield trait (Alemu *et al*, 2021). To overcome these challenges, the introduction of productive, yet tropically adapted chicken strains provides an opportunity to increase meat yield and egg number in developing countries (Alemu *et al*, 2021).

The African Chicken Genetic Gains (ACGG; https://africacgg.net/) project led by the International Livestock Research Institute (ILRI) was started in 2014 and implemented over five years in Ethiopia, Tanzania and Nigeria. The programme's vision was to increase smallholder chicken production and productivity growth as a pathway out of poverty in sub-Saharan Africa. To increase meat yield and egg number in a sustainable manner, selected chicken strains were made available to rural farmers in Ethiopia, Nigeria and Tanzania, through the ACGG project (Alemu *et al*, 2021). Sasso and Kuroiler chickens were among the improved strains that had been tested in Tanzania and were distributed on a large scale by the ACGG project. These strains are suitable for backyard systems and can be used for meat and egg production, with minimal provision of commercial feed (Dessie and Getachew, 2016). Following the success of the ACGG project, dual-purpose tropically adapted chicken breeds such as Hubbard, Kuroiler, Noiler and Sasso are being promoted by ILRI under the Tropical Poultry Genetic Solutions (TPGS) project (https://www.ilri.org/research/projects/tpgs). The Ghana component of TPGS is collaborating with local stakeholders to test the performance of the newly selected strains in terms of body weight, egg production and survivability in two different agroecological zones compared to the indigenous chicken in Ghana. The superior performance of introduced strains such as Kuroiler and Hubbard over indigenous chickens will give smallholder farmers a good alternative for backyard poultry production. This should help improve income generation and livelihood of smallholder farmers in Africa and contribute to the attainment of the UN Sustainable Development Goals (SDGs), particularly SDG 1 (No poverty), SDG 2 (Zero hunger) and SDG 3 (Good health and well-being). Therefore, the objective of this research was to evaluate the performance of the TPGS-introduced chicken genotypes in terms of survivability, growth and egg production as well as their acceptance by stakeholders, particularly farmers. The findings of this study will serve as valuable input for chicken breeders, policymakers and other stakeholders in the development of selected more resilient chicken genotypes in Africa.

Materials and methods

Project scope and farmer selection

The Ghana component of the TPGS project, managed by the Animal Science Department of the University of Ghana with support from the Ministry of Food and Agriculture (MoFA), supplied Kuroiler and Hubbard chickens to selected poultry farmers across two management systems – intensive and semi-intensive – in the forest and coastal savannah agroecological zones of Ghana. The Kuroiler is a tropically adapted dual-purpose chicken developed in India by Kegg Farms Private Limited. It is widely reported that this cross-bred chicken originated from crossing Rhode Island Red, White Leghorn, Barred Plymouth Rock, and two Indian local chicken breeds with some broiler blood infusion to obtain specific broiler characteristics (Dessie and Getachew, 2016). Hens attain 2.5kg within 12 months, begin laying eggs at five to six months, and then lay 150–200 eggs during their 12–16-month egg-laying period, initially more than 20 eggs per month (Dessie and Getachew, 2016). Males reach 4kg in 12 months and weigh at least 1kg at around three months. The breed thrives well on household waste, scraps and vegetation and, thus, does not compete with human food for grain or require any special feeding (Dessie and Getachew, 2016). Hubbard has been a worldwide reference for broiler breeding stock, with the company supplying day-old grandparent and parent stock chicks all over the world. Hubbard chickens are characterized by good growth, feed conversion ratio, excellent viability and good meat yield. The Hubbard JA57 reach between 2.3 and 2.4kg on average at 20 weeks (https://www.hubbardbreeders.com/).

The present project was undertaken in the Greater Accra, Central, Eastern, Bono and Ashanti Regions of Ghana (Figure 1), the main areas of chicken production in the country. Stakeholder engagement, particularly with the Poultry Farmers Association, Regional and District Livestock Officers of the MoFA and the Women in Poultry Value Chain, enabled us to identify and agree with the targeted beneficiaries their needs and challenges in adopting improved technologies to enhance their production efficiency. Two service providers located in the Eastern and Ashanti Regions of Ghana were contracted to hatch fertilized eggs and brood the chickens for a period of five weeks before they were distributed to the participating poultry farmers. The study sampled poultry farmers from five regions across two agroecological zones in Ghana. These farmers were provided with Kuroiler and Hubbard chicken genotypes, which had been selected for improved growth and egg production performance, to raise under semi-intensive or intensive systems. In these systems, housing, feeding and veterinary care were provided for the chickens, with birds allowed to freely roam around the farm during the day in the semi-intensive system. Six communities (villages/towns) were sampled from each of the selected regions. Within each community, nine households (smallholder farmers) were selected as project beneficiaries. Thus, 54 households per region and 270 farmers nationwide participated in the programme. Three enumerators with minimum qualifications that met the standard selection criteria by ILRI were chosen in each region to support the project in terms of data collection. The Regional Livestock Officers of MoFA in the participating regions were assigned as supervisors to oversee the activities of the enumerators. Enumerators and their supervisors were trained on the data collection protocols, which were captured in real time using tablets installed with ODK software (ICTD, 2010). Throughout the project, regular communication was established between enumerators and the project team to ensure speedy resolution of any challenges encountered in the field.

Egg incubation and mothering of chickens

In total, 10,080 fertile Kuroiler eggs were imported from Uganda on 6 December 2021 and hatched by Akro Farms, Akropong, Eastern Region, one of the selected private hatcheries whilst a second batch of 5,040 fertile eggs each of the Hubbard JA57-I and JA57-II strains were received from Morocco on 9 April 2022 and hatched by Topman Farms, Kumasi, Ashanti Region. The day-old chicks were raised intensively in mother units for up to five weeks. The feed provided was a commercially prepared chick mash with 3,080kcal/kg of energy and 21% crude protein. All prescribed prophylactic vaccines, including the 1^{st} and 2^{nd} Newcastle and Gumboro vaccinations, were provided. Two hundred and seventy (270) beneficiary farmers received 40 birds of each of the three chicken strains at random, with no special preference for sex and size.

Data collection and analysis

Data on body weight, age at first egg (AFE), egg number and weight were collected from participating farmers using ODK tools. The first phase of TPGS Ghana took place between December 2021 and March 2023. During this period, on average, poultry farms were visited monthly for sensitization and body weight measurements, starting when the birds were five weeks of age for approximately 8 visits in total. Data entries were double-checked by supervisors before the final authorization to upload onto the server at ILRI. A total of 180 eggs made up of 60 eggs each from Kuroiler, local forest and local coastal savannah chicken ecotypes aged 34–36 weeks, were randomly sampled from farmers and kept at room temperature for three days before measuring egg quality parameters including egg weight, Haugh unit, albumin height and yolk colour using an electronic egg analyzer (ORKA Food Technology LLC, 2006) at the Molecular Genetics Laboratory, Department of Animal Science, University of Ghana, Legon. Shell thickness was measured with an electronic Vernier calliper by taking the average thicknesses of the large, central and narrow ends of individual eggs (Melesse *et al*, 2010).

The one-way analysis of variance (ANOVA) procedure of GenStat 12th Edition (2009) was used for the data analysis. The effect of age and genotype of chicken were estimated based on the following model:

$$y_{ijk} = \mu + a_i + g_j + ag_{(ij)} + e_{ijk}$$

Where y_{ijk} = body weight of chicken; μ = the population mean; a_i = effect of age of chicken; g_j = effect of genotype; $ag_{(ij)}$ = age and genotype interaction; e_{ijk} = residual or the random error term.

The effect of management system and sex on growth performance of Kuroiler chickens at week 17 was analyzed based on the following model:

$$y_{ijk} = \mu + m_i + s_j + ms_{(ij)} + e_{ijk}$$

Where y_{ijk} = body weight of chicken; μ = the population mean; m_i = effect of management system; s_j = effect of sex; $ms_{(ij)}$ = management and sex interaction; e_{ijk} = residual or the random error term.

The effect of genotype on egg quality traits of sampled eggs of Kuroiler and two local chicken ecotypes from the forest and coastal savannah zones of Ghana was analyzed using the following model:

$$y_{ij} = \mu + g_i + e_{ij}$$

Where y_{ij} = egg quality parameter; μ = the population mean; g_i = effect of chicken genotype; e_{ij} = residual or the random error term.

Where the ANOVA indicated a significant genotype effect, the means were separated using Tukey's test at the 5% margin of error. Tukey's test was chosen for mean separation on account of its robustness, requiring a single value judging the significance of all mean differences and is thus quick and easy to use (Steel and Torrie, 1981). We also surveyed farmer trait preferences in the introduced chicken genotypes. Farmers were asked to rank ten traits (fast growth, good survivability, good disease resistance, low feed intake, laying more eggs, laying big eggs, good mothering, plumage colour, tough meat and soft meat) indicating their preference for the introduced chicken genotypes compared to local chickens. In terms of hatchability, we used the two-proportion Z-Test to calculate the true difference in the hatching performance percentages between Kuroiler and Hubbard chicken eggs.

Results and discussion

Body weight and growth performance

The growth rates of both the Kuroiler and Hubbard chicken genotypes are shown in Figure 2. The growth rates of both the Kuroiler and Hubbard chickens were superior to those of the local chickens of Ghana kept under semi-intensive management reported in an earlier study (Osei-Amponsah *et al*, 2012). This finding of superior growth performance and productivity of the introduced chicken genotypes compared to the local chickens aligns with previous studies that have reported on the benefits of using adopted breeds selected for improved production in smallholder systems (Bamidele

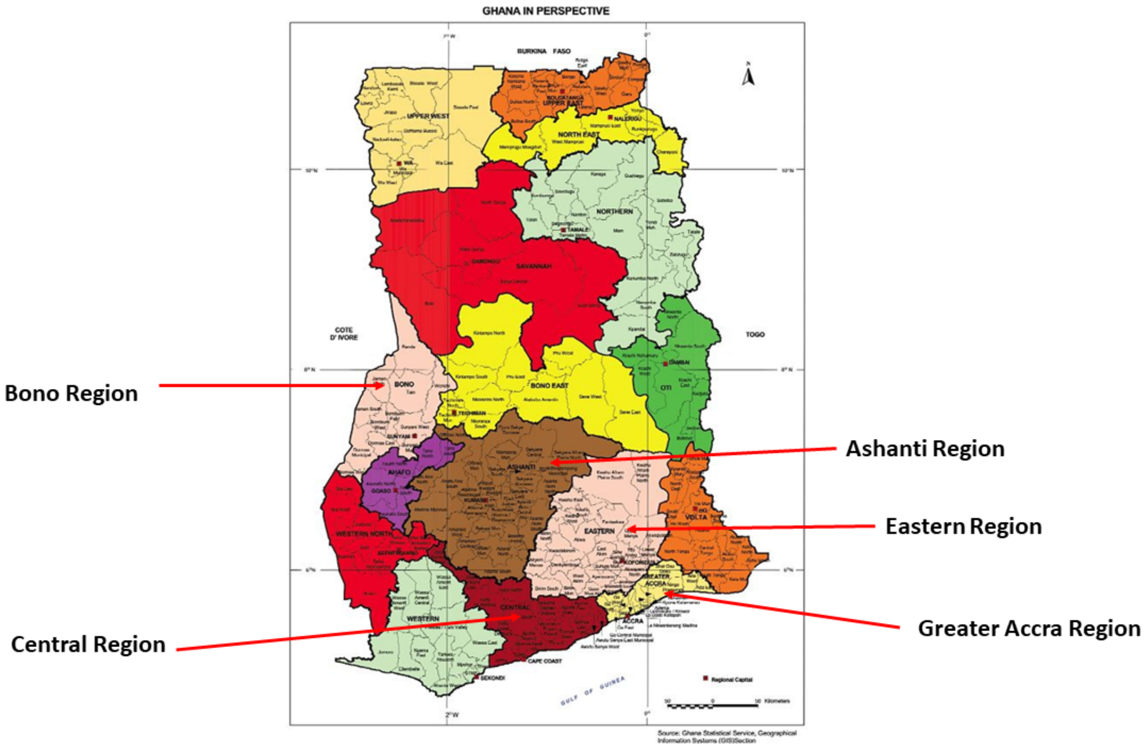

Figure 1. Map of Ghana showing sampled regions. Source: Ghana Statistical Service, Geographical Information Service (GIS) Section.

et al, 2023). The significant reduction in the number of sampled chickens over time was largely due to the disposal (sale) of particularly male birds after 17 weeks to reduce the feeding costs of the farmer.

The production system was found to influence the growth of chickens. On average, Kuroiler chickens raised intensively weighed significantly ($p < 0.05$) more (2.0kg) than those kept under semi-intensive (1.8kg) management (Table 1). The significant ($p < 0.05$) effect of the production management system on growth was expected because birds in confinement spend less energy moving around than those in the open. Furthermore, husbandry practices, such as feeding, biosecurity and veterinary care, are expected to be better and easier in confinement. In a previous study on growth performance of chickens, Osei-Amponsah *et al* (2012) reported the average body weight (g) of female and male local Ghanaian chickens at hatch, 20 and 28 weeks to be 25.8, 1,033.5 and 1,273.5g, respectively. In the male population, the average body weights at these ages were 26.6, 1,358.5 and 1,593.5g respectively indicating a clear sexual dimorphism. This growth performance in local chickens was however significantly ($p \leq 0.05$) lower than the exotic Sasso T44 raised under the same management condition which gave average body weights in females at the same ages as 38.2, 2355.0 and 2,635.0g with corresponding values of 37.6, 2,777.0 and 3,192.0g in males (Osei-Amponsah *et al*, 2012).

The body weights recorded for Kuroiler chickens in the current study were lower than those reported for the same breed and age on station in Ethiopia (Alemu *et al*, 2021; Bedru, 2021). This emphasizes the importance of improved management practices for the growth and development of chickens. Local chicken improvement programmes should incorporate supplementary feeding, veterinary care and housing to enhance productivity (Osei-Amponsah *et al*, 2012). Under improved management, local Ghanaian Savannah and Forest chicken ecotypes weighed between 1.2–1.7kg at seven months of age (Osei-Amponsah *et al*, 2012). This was lower than that recorded at four months for both Kuroiler and Hubbard chickens in the present study. The superior growth performance of Kuroiler over local chickens is due to the genetic potential of the breed as a commercial strain (Dessie and Getachew, 2016), as it has been bred for superior growth through selection and strain crossing. This finding of considerable differences in live body weight among chicken strains is in line with those of previous studies (Mulugeta *et al*, 2020; Alemu *et al*, 2021; Guni *et al*, 2021; Kassa *et al*, 2021). Sasso chickens have also been reported to be superior in growth compared to Koekoek and Sasso-RIR crosses in Ethiopia and were recommended for semi-scavenging production systems (Fekede *et al*, 2021). There is thus a need for the selection and improvement of the growth potential of local chickens to take full advantage of their adaptive potential and genetic variability with respect to various environmental challenges (Osei-Amponsah *et al*, 2013). Our findings indicated a significant ($p \leq 0.05$) effect of management system on age at first egg in Kuroiler chickens. Kuroiler chickens raised under intensive management laid significantly earlier (22.3 weeks

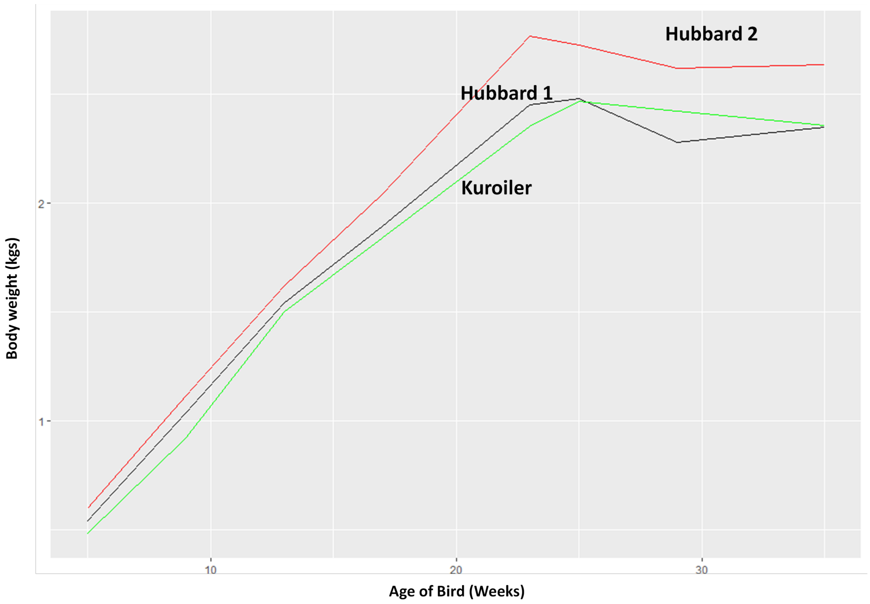

Figure 2. Growth performance of experimental chickens

old) than those raised under semi-intensive management (25 weeks old). Additionally, Kuroiler chickens raised under intensive management grew significantly ($p \leq 0.05$) faster than those under semi-intensive production systems (Table 1).

Egg production performance

The egg production performance of the Kuroiler and the two Hubbard lines was also superior to that of local Ghanaian chickens. The age at first egg (AFE) for the Hubbard birds was 17.5 weeks compared to 28 weeks for the local chickens, and the expected egg number and egg weight (Figure 3) of the Hubbard chickens increased with age.

We further compared the hatching performance of fertilized Kuroiler and Hubbard eggs under Ghanaian conditions, based on the data received from the hatchery operator and shown in Table 2. Both genotypes recorded a relatively high percentage hatched on fertile ($\geq 83\%$). Egg hatchability is affected by egg physiology, egg size, environment (bird nutrition and handling of eggs), bird genotype, genetic diversity and their interactions. Hence, the current results could not be solely associated with the genetic makeup of birds. The hatchability percentages of 83 and 85% of incubated Kuroiler and Hubbard eggs, respectively, and can be considered satisfactory, although a higher hatchability of 87.8% has been reported for indigenous chickens in the forest and savannah zones of Ghana (Osei-Amponsah *et al*, 2014). Comparison of hatching performance based on the proportion Z-Test indicated significantly ($p < 0.001$) higher fertility of Hubbard chicken eggs compared to Kuroiler eggs (Table 2), which can be attributed to variations in egg storage and handling by the two hatchery operators.

Egg production performance of Kuroiler and the two Hubbard lines studied was superior to that of local chickens kept under similar management conditions in Ghana (Osei-Amponsah *et al*, 2015). Kuroiler birds raised intensively under on-station management in Tanzania had an age at first egg of 22.7 weeks whilst 24.8 weeks was achieved for on-farm semi-scavenging management. In other studies, egg production traits of chickens selected for fast growth (Sasso, Bovans and Koekock) were generally higher than those of local chickens, with variations observed across the genotypes (Guni *et al*, 2021). The fact that chickens in the present study began laying from week 23 may imply an adaptation strategy for survival under semi-intensive production conditions. Egg production is very demanding of nutrients; therefore, under free range, it is likely that the bird must first build its reserves adequately before engaging in this nutrient-demanding activity and hence adapt to start later rather than earlier. The mean egg weight, albumin height and Haugh unit of Kuroiler chickens were all significantly ($p \leq 0.05$) heavier than those of the indigenous birds; however, the shell thickness and yolk colour were not significantly different (Table 3).

The relatively higher egg weights obtained for Kuroiler chickens compared to local chickens can be attributed to genetic differences. Both albumin height (AH) and Haugh unit were positively and significantly related to egg weight (Moula *et al*, 2013; Osei-Amponsah *et al*, 2014; Bekele *et al*, 2022). Kuroiler eggs in this study were superior to the eggs from local chickens in terms of average albumin height and Haugh unit, similar to their mean egg weight. Previous findings indicate that local chicken eggs have relatively stronger shell and yolk ratios than Sasso T44. However, the egg quality of local chickens is lower than that of Sasso T44 chickens because of the negative correlation between yolk ratio and AH, the main determinant of egg quality (Osei-Amponsah *et al*, 2014). Haugh unit

Table 1. Range and mean body weight of Kuroiler chickens (±SE) by management system and sex at week 17. Within-rows means followed by different superscripts are significantly ($p \leq 0.05$) different.

		Intensive	**Semi-Intensive**
Cockerels (Males)	Range (g)	1,000–3,940	700 – 3,920
	Mean (g)	2,231.65^a±29.63	1,851.2^b±28.33
	N	536	530
Pullets (Females)	Range (g)	920–4,000	610–3,630
	Mean (g)	1,799.41^a±18.35	1,696.55^b±23.04
	N	619	540
All	Range (g)	920–4,000	610–3,920
	Mean (g)	2,005.2^a±18.12	1,806.79^b±18.63
	N	1,155	1,070

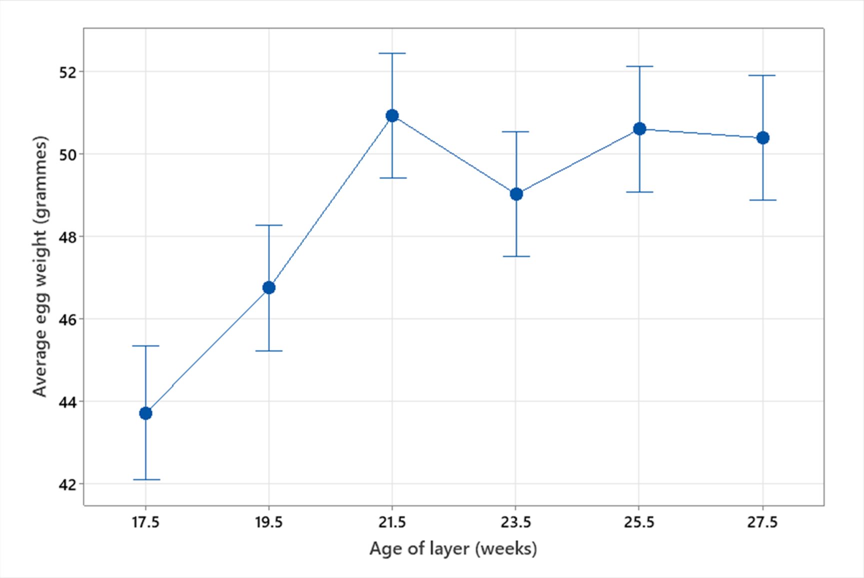

Figure 3. Variation of egg weight of Hubbard chickens by age of bird under a semi-intensive system in Ghana

Table 2. Hatching performance of introduced chicken strains. *, the hatching performances of both Hubbard lines were computed by the hatchery operator together. #, significant proportional differences (Z statistics) are indicated by $p \leq 0.05$. Within rows, proportions followed by different superscripts are significantly different ($p \leq 0.05$).

Parameter	**Chicken strains**		**Z Statistic**	**p-value** $^{\#}$
	Kuroiler	**Hubbard***		
Eggs received (N)	10,080	10,080		
Eggs set (N)	9,980	9,427		
% Egg set	99^a	93^b	20.54	< 0.001
Fertile eggs (N)	8,700	8,671		
% Fertile	87^b	92^a	-10.92	< 0.001
Infertile (N)	1,280	750^b		
% Infertile	13^a	8	11.08	< 0.001
% Hatched on fertile	83^b	85^a	-3.59	0.0003
% Hatched on set	73^b	78^a	-8.09	< 0.001

Table 3. Egg quality trait parameters of Kuroiler and local chicken ecotypes of Ghana. Within-rows means followed by different superscripts are significantly ($p \leq 0.05$) different.

Parameters	N	Range	Kuroiler	Forest	Coastal Savannah
Egg weight (g)	180	32.2–101.8	$88.8^{a}\pm9.42$	$58.70^{b}\pm9.9$	$52.50^{c}\pm10.3$
Albumin height (mm)	180	2.1–5.5	$3.94^{a}\pm0.68$	$3.54^{a}\pm0.93$	$3.10^{b}\pm0.52$
Shell thickness (mm)	180	0.24–0.48	0.39±0.05	0.38±0.04	0.38±0.04
Yolk colour	180	1.0–11.0	5.80±2.01	6.27±2.26	6.43±2.79
Haugh unit	180	43.3–97.6	$77.30^{a}\pm9.85$	$73.40^{a}\pm9.05$	$66.40^{b}\pm11.7$

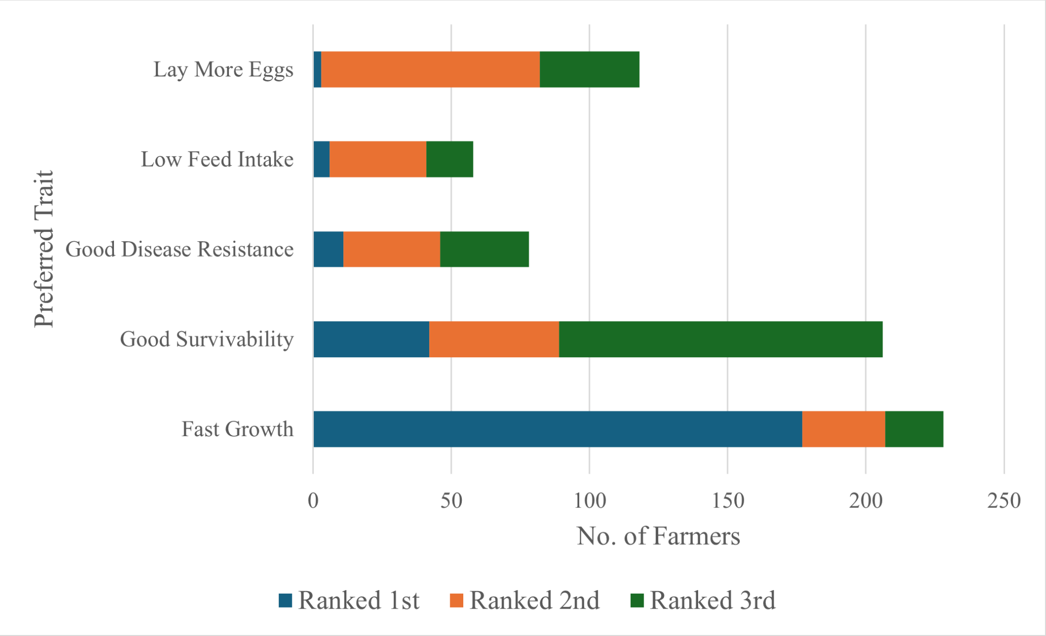

Figure 4. Distribution of farmers' most preferred traits in chickens

of eggs from the local chickens was lower than that of exotic chickens (Osei-Amponsah *et al*, 2014; Kejela *et al*, 2019). The mean Haugh unit of local chickens in Ghana ranged from 64.97 to 67.34, lower than that of naked-neck and normal-feathered chickens in Nigeria, which were 73.22 and 71.40, respectively (Yakubu *et al*, 2008; Osei-Amponsah *et al*, 2014).

Farmer perception and acceptance of introduced chicken genotypes

In terms of trait preferences, farmers were asked to rank ten traits (fast growth, good survivability, good disease resistance, low feed intake, laying more eggs, laying big eggs, good mothering, plumage colour, tough meat and soft meat). Fast growth, good survivability, good disease resistance, low feed intake and increased egg number are the most preferred traits in this study (Figure 4). The result of 'fast growth' and 'increased egg number' was expected. This is because, smallholder poultry farmers in sub-Saharan Africa mainly keep chickens for their meat and eggs hence these traits are of much importance to them (Sonaiya and Swan, 2004; Ochieng *et al*, 2013; Melesse, 2014). The 'good survivability' and 'good disease resistance' trait preferences are consistent with previous studies (Faustin *et al*, 2010; Terfa *et al*, 2019). Diseases in all poultry enterprises can lead to huge economic loss hence farmers' choice of 'good survivability' and 'good disease resistance' is not surprising (Dar *et al*, 2018).

Beneficiary farmers provided positive accounts of experimental birds. They attested to the high performance of both Kuroiler and Hubbard in terms of their body weight, egg size and egg number compared to the local chicken. The socioeconomic gains and prestige these birds have brought to them in their communities were emphasized. Almost all beneficiary farmers mentioned income generation through the sale of larger and more eggs as well as cockerels. The adaptation abilities of the introduced birds were evidenced by the relatively low mortality and their resilience to environmental and farmer management conditions. This led to non-beneficiary poultry farmers expressing interest in the TPGS project and requesting inclusion in any future initiatives. The farmers showed a strong preference for the introduced breeds, as they were found to be more market oriented (Bamidele *et al*, 2023). Furthermore, the use of improved tropically adapted chicken breeds in smallholder flocks in sub-Saharan Africa led to increased production and productivity, generating more income while contributing to food security and social and ecological resilience (Birhanu *et al*, 2023). As highlighted in the literature, livelihood enhancement programmes often provide beneficiaries with poultry birds and feed to contribute towards food security and income generation (Singh *et al*, 2022).

Conclusion

This study's intervention of introducing improved, dual-purpose chicken breeds to smallholder farmers aligns with previous findings, demonstrating the potential to significantly enhance livelihoods through the adoption of better-suited poultry genetics. The fast-growing and productive nature of the introduced chicken strains, particularly in terms of egg production, suggests a high likelihood of acceptance among poultry farmers in Ghana. Although the introduced breeds are more effectively managed under intensive systems, they have also shown resilience and satisfactory performance in semi-intensive and extensive production systems. These findings, along with supportive evidence from other studies, underscore the potential of these dual-purpose chickens to support faster income generation for smallholder farmers under prevailing production conditions in Ghana. The introduction of adapted, dual-purpose chicken genotypes, such as the Kuroiler and Hubbard chickens has shown promising results in enhancing productivity and market-oriented performance. This study thus provides valuable insights for stakeholders to make informed decisions on local chicken breed diversification, conservation and improved production through efficient management, breeding and nutrition practices. The government and relevant stakeholders should work to ensure that tropically adapted chicken strains, selected for improved production, are made widely accessible to smallholder farmers. This can significantly boost poultry production and income generation across rural farming communities. Future initiatives should include comprehensive economic assessments, particularly cost-benefit analyses, to evaluate the long-term profitability and sustainability of the introduced chicken breeds. Such data will guide strategic genetic improvement programmes and investments in the poultry sector. To maximize the benefits of the new chicken strains, farmers should be trained in efficient management practices, including breeding, feeding and health care. This will boost productivity and profitability of the introduced poultry under varied production systems. The Ministry of Food and Agriculture should develop supportive frameworks that promote the distribution and management of the introduced chicken lines as well as financial and technical support, alongside market linkages, to enhance the success of TPGS and similar interventions.

Authors contribution

Richard Osei-Amponsah: conceptualization, supervision, data analysis, preparation of initial draft, review and approval of final draft. Ricky Aboagye Poku: data collection, review and approval of final draft. Ebenezer Agyemang Duah: Data collection, data analysis, review and approval of the final draft. Augustine Naazie: supervision, data analysis, review and approval of the final draft. Raphael Ayizanga: data collection, data analysis, review and approval of final draft. Harrisson Njamba: data collection, data analysis, review and approval of final draft. Wondmeneh Esatu: sourcing of fertilized eggs, review and approval of final draft. Mulugetta Y. Birhanu: sourcing of fertilized eggs, review and approval of the final draft. Tadelle Dessie: Conceptualization, fund acquisition, review and approval of the final draft.

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Conflict of interest statement

The authors declare no conflicts of interest.

Data availability

The authors affirm that all data necessary to confirm the conclusions of the article are presented within the article, figures and tables. Raw data analyzed for this article are available from the corresponding author.

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The EURISCO-EVA Information System, an innovative approach to the data management of multi-site crop evaluation data

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Abstract: This paper introduces EURISCO-EVA, an extension of the European Search Catalogue for Plant Genetic Resources (EURISCO) hosted at and maintained by the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben on behalf of the European Cooperative Programme for Plant Genetic Resources (ECPGR). This information system facilitates standardized data collection, sharing and analysis for plant genetic resources for food and agriculture (PGRFA) characterization and evaluation. In the framework of the European Evaluation Network (EVA), public–private partnerships aiming at the evaluation of crop accessions conserved in European genebanks, EURISCO-EVA provides a standardized data repository for multi-site evaluations of different crops. Through centralizing metadata maintenance, EURISCO-EVA ensures uniformity in trait definitions, experimental designs and passport data, promoting the efficient exchange of observed phenotypic data. EURISCO-EVA currently stores more than half a million phenotypic data points for 4,845 PGRFA accessions from 6 genera and 17 species, collected through 382 phenotypic experiments conducted at 115 experimental locations across 33 countries, involving 89 project partners. This platform offers a user-friendly web interface, empowering its users with features such as map-based filtering of trial locations, statistical overviews and customizable reports. EURISCO-EVA's robust administrative functionalities, coupled with standardization efforts, enhance data quality and harmonization, providing a robust and scalable system for storage of and access to crop evaluation data that could be further enhanced by adding analysis modules. EURISCO-EVA also formed the basis for the data management of two research projects (AGENT and INCREASE) under the European Union Horizon 2020 funding programme, providing the background organization of complex datasets used to address future challenges in European agriculture.

Keywords: Crop evaluation, Genebank, Information system, Plant Genetic Resources, Metadata

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Introduction

In the coming decades, a growing population, climate change and the need to protect ecosystems will create new challenges for agriculture and global food security. To address these challenges, sustainable farming and increased crop production are required. Developing crop varieties with resilient traits like disease resistance, drought and heat tolerance will be crucial to achieving these goals (McCouch *et al*, 2013; Pixley *et al*, 2023). This requires access to a diverse pool of plant genetic resources for food and agriculture (PGRFA) conserved *ex situ* by genebanks to identify and incorporate valuable traits into new crop varieties (Sanchez *et al*, 2023; King *et al*, 2024).

The accessibility of these PGRFA accessions and their related passport, characterization and evaluation data, is strictly linked with the existence and updating of information systems. This involves gathering data for acces-

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sions from many germplasm collections and projects into centralized sources, facilitating a smooth flow of germplasm material and data among institutions. Therefore, developing an information system that collects data from various sources in a standardized format and creates searchable datasets on genetic resources is key to PGRFA access and sustainable use (Khoury *et al*, 2010; Guzzon and Ardenghi, 2018; ECPGR, GenRes Bridge Project Consortium, ERFP & EUFORGEN, 2021). A standardized phenotypic data platform is essential for interoperability, enabling integration and comparison of data from diverse sources, allowing also for the feeding and operation of international information systems on plant genetic resources (PGR) (Weise *et al*, 2020). A standardized information system promotes reproducibility and validation of research findings, fostering transparency and collaboration among researchers. Standardization streamlines data integration and analysis processes, improving efficiency and reducing duplication of effort and resources, allowing the implementation of the FAIR (Findable, Accessible, Interoperable, Reusable) data principles (Wilkinson *et al*, 2016; Papoutsoglou *et al*, 2023). Data standardization ensures also data quality and integrity by defining clear guidelines for collection, storage and validation. In this framework, MIAPPE (Minimum Information About a Plant Phenotyping Experiment) provides a community data standard for the plant phenotyping domain (Krajewski *et al*, 2015; Papoutsoglou *et al*, 2020).

To exploit the genetic wealth of PGR conserved *ex situ* in genebanks, multi-site pre-breeding characterization and evaluation of PGRFA are fundamental to inform and speed up the complex process of crossing, selection and testing of plant material needed to produce a new elite cultivar and preparing material ready to be incorporated into crop breeding programmes (Cockel *et al*, 2022). In Europe, the European Evaluation Network (EVA) for PGRFA (http://www.ecpgr.org/eva/), coordinated by the European Cooperative Programme for Plant Genetic Resources (ECPGR), is an international initiative aimed at increasing the use of crop genetic diversity as well as the diversity of stakeholders in plant breeding. In joint efforts with partners from both public and private sectors, EVA is producing standardized evaluation data for various crop cultivars and landraces found in European genebanks. This data includes both phenotypic characteristics and genotypic information, which can be used to identify suitable breeding materials and genetic markers for relevant traits. EVA operates through specific networks tailored to different crops, including cereals and vegetables. Initially established as five different crop networks (carrot, lettuce, maize, pepper, and wheat and barley), ECPGR launched a new EVA network on legumes in 2024, which covers seven different crop groups: chickpea, common bean, faba bean, lentil, lupin, pea and orphan legumes, thus vastly expanding the project partnership. EVA provides an opportunity to promote the sustainable use of PGRFA to facilitate the adaptation of European agriculture to climate change and to contribute towards achieving related Sustainable Development Goals.

In this paper we describe the EURISCO-EVA Information System, which was developed as an extension of the European Search Catalogue for Plant Genetic Resources (EURISCO; see Weise *et al* (2017); Kotni *et al* (2023)) and is a service provided by ECPGR to the PGRFA user community. The system provides partners with a central data repository and allows the collection of standardized phenotypic data in the framework of EVA. It features filter and display options and can facilitate the publication of datasets after the project embargo through integration with EURISCO. EURISCO-EVA served as a blueprint for data management infrastructures developed in other European projects like AGENT (https://agent-project.eu/) and INCREASE (https://www.pulsesincrease.eu/). By adopting a common model and protocols, these projects standardize and thereby facilitate the exchange of data and information among different databases and systems. This interoperability enhances collaboration and coordination among various stakeholders involved in PGRFA conservation and breeding efforts across Europe and beyond.

The EURISCO-EVA Information System

Database content

The EURISCO-EVA database currently stores the data of five crop networks: carrot, lettuce, pepper, maize, and wheat and barley. The wheat and barley network accounts for three different crops, barley (*Hordeum vulgare* L.), durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) and common wheat (*Triticum aestivum* L.). In the lettuce network, data on wild prickly lettuce (*Lactuca serriola* L.) are stored together with data on cultivated lettuce (*Lactuca sativa* L.). In the pepper network, five species are considered: *Capsicum annuum* L., *C. baccatum* L., *C. chacoense* Hunz., *C. chinense* Jacq., *C. frutescens* L. At the time of writing, these five networks thus cover a total of eight crops accounting for a total of 4,845 accessions and 282 phenotypic traits with data. The 89 network partners work in 33 countries, carrying out characterization and evaluation activities in 115 experiment locations. As of June 2024, more than 500,000 total phenotypic data points have been collected in 382 phenotypic trials and this number is continuously growing. Table 1 provides an overview of the data of the different crop networks and Figure 1 presents a summary of the data points, phenotypic trials, traits and evaluated accessions by country as of June 2024.

In addition to phenotypic evaluation data, EURISCO-EVA stores relevant metadata such as accession passport data, trait and method definitions, information on phenotypic trials, network partners and genotyping experiments. A new record for any of the metadata can be created or the existing record can be updated as needed. A new partner can join an existing network; however, they will only have access to the network's

Table 1. Summary overview of the data available on EURISCO-EVA of the current five EVA networks (as of 11 June 2024). Countries of operation are those where trials are performed, experiment locations refer to locations within countries.

	All Networks	Carrot	Lettuce	Maize	Pepper	Wheat & Barley
Crops	8	1	2	1	1	3
Accessions evaluated	4,845	67	291	861	181	3,445
Partner institutes	89	14	12	18	15	47
Countries of operation	33	8	8	9	13	25
Experiment locations	115	14	6	30	10	58
Phenotyping experiments evaluated	382	27	13	63	15	264
Traits evaluated	282	138	21	51	26	46
Phenotypic data points	510,097	88,199	10,217	90,359	19,327	301,995

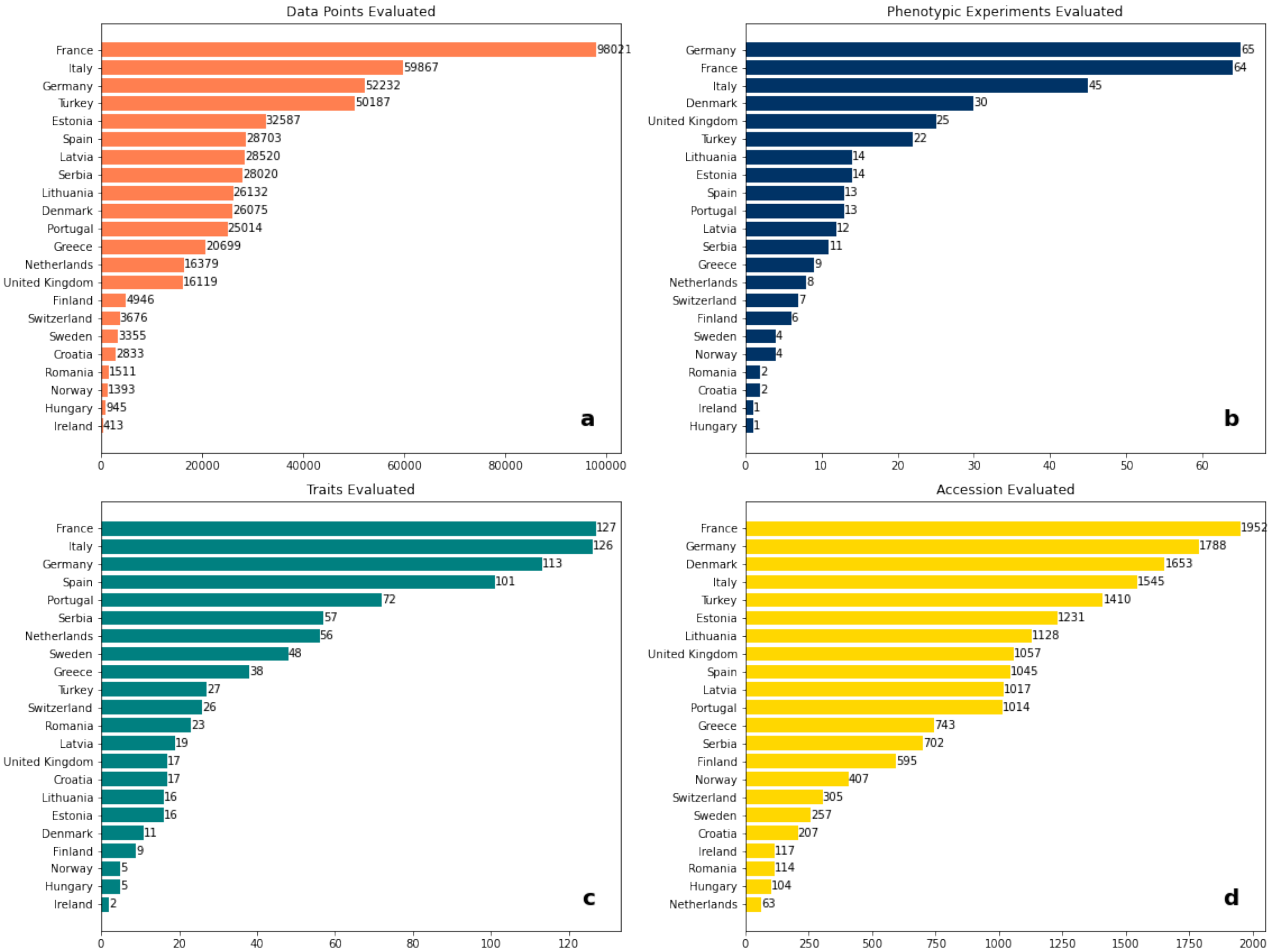


Figure 1. Summary of evaluation data and metadata in EURISCO-EVA for all crop networks disaggregated by evaluation countries. a) Total number of evaluation data points obtained by country, b) Total number of phenotypic trials evaluated by country, c) Total number of traits evaluated by country, and d) Total number of accessions evaluated by country (data as of 11 June 2024).

data created on or after their joining date. The EVA accessions' passport data follow the Multi-Crop Passport Descriptors standard (Alercia *et al*, 2015) along with some EVA-specific identifiers, e.g. material type (original accession, single-seed descent line, cross or check), EVA ID, male and female parent for crosses, and parent DOIs, where applicable. These project-specific parameters allow categorization of the accessions in the network and can be adjusted centrally by the EVA coordinator. Each trait is defined by a unique trait acronym, its trait name, a detailed trait method description, a Crop Ontology term, trait group, measurement unit and allowed scores. The allowed trait scores are of two types, metric and rating scores, and define the range of allowed values or the allowed scoring entries, respectively. Traits can be further grouped into different categories, such as morphological, agronomic, quality or (a-)biotic stress traits, to facilitate searches and filtering. The trial definition broadly consists of the trial location (ideally by GPS coordinates), experimental and field design as well as meteorological and soil conditions, which are important parameters when comparing data from multiple locations. The evaluation data are stored as trait scores that were observed for the accessions under defined trial conditions. The system can also host additional data like accession images and links to genotypic data repositories. For every partner involved in the project, there is information on the location of the organization and the trials as well as contact information of the responsible persons.

Technical requirements, features and technology

The EURISCO-EVA infrastructure was developed as an extension of the European Search Catalogue for Plant Genetic Resources (EURISCO), operated by the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Germany, on behalf of and under the supervision of ECPGR, providing additional necessary functionality beyond the existing EURISCO infrastructure in the framework of the abovementioned EVA network. The EURISCO-EVA Information System is also maintained on behalf of ECPGR and has been developed by IPK. It has been available online to the EVA network partners since 2022 and its management is handled by the ECPGR EVA coordinator.

The primary access point to EURISCO-EVA is its web interface (https://eva.ipk-gatersleben.de/), developed using the Oracle Application Express (APEX) technology, version 21. Partners can use their login credentials to access their network and view their network's data collected on or after their joining date. There are two entry points: a general homepage and a main page for each crop network. After logging in, users first access the common homepage, which introduces the EVA project, offers user manuals, provides downloadable templates (see Supplemental data 1 and 2) for recording or uploading data, and includes general information about the data and metadata stored in the system.

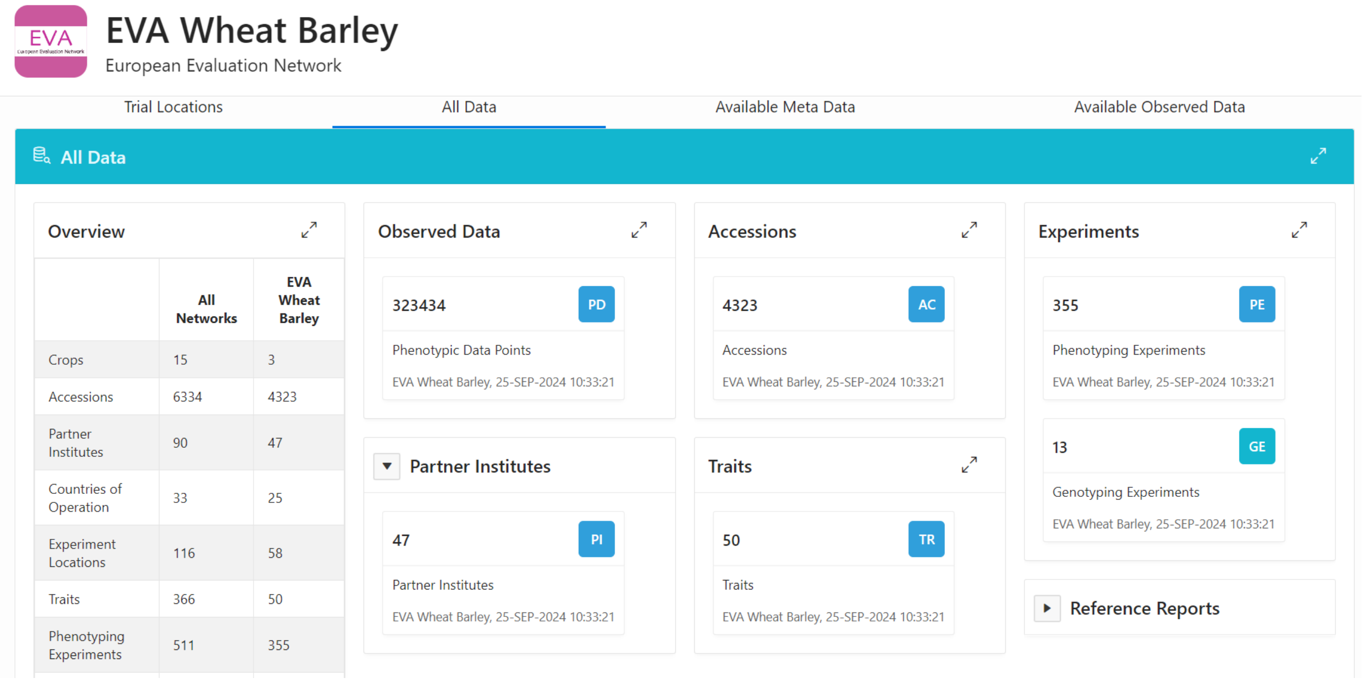
Every crop network has its homepage with the same type of information, accessible to the network partners only. The network homepage has four tabs (Figure 2). The first tab 'Trial Locations' shows a map giving users an overview of the geographic diversity of the experimental trial locations. The data on the map can be filtered and searched using one or more available filters, such as crop, year, experiment group, organization, country or trial ID. The second tab 'All Data' provides on the left-hand side a statistical overview of available data for the crop network and the total data of all crop networks (Figure 2). Moreover, it has several cards that act as hyperlinks to generate reports on accession passport and phenotypic data, partner information, specifics of the phenotypic traits as well as phenotyping and genotyping experiments. The third tab 'Available Metadata' provides numerical information on relevant available metadata, including passport data, trial details, trait definition and network partner details, already grouped by important parameters such as accession material type or trait group. The hyperlinks in the statistical reports open a filtered report corresponding to the selected parameter. The fourth tab 'Available Observed Data' displays phenotypic data already grouped by crop or species, predefined experiment group or year as well as country of origin of the accessions and institutes maintaining the material.

The user interface of this application is designed to offer multiple methods for retrieving and filtering data to suit different needs and preferences. Below is a detailed overview of these methods and how they work:

1. Default search and filter options: The default search feature allows users to quickly find information by entering keywords or criteria into a search bar. For example, if a user is looking for data on 'Genotype A,' they can simply type this term into the search bar, and the system will display all relevant records containing the string 'Genotype A.' Additionally, users can apply basic filters from a predefined set of options such as date ranges or data categories to narrow down the search results.

2. One-click filters: For more common searches, the interface provides one-click filters. By clicking on them, users can instantly retrieve the data without having to apply a filter manually. For example, to retrieve phenotypic data specifically for wheat, simply click on 'Wheat' in the one-click filter menu labelled 'Grouped by Crop'.

3. Advanced searches with interconnected drop-down filters: The advanced search functionality provides a more detailed and precise approach to data retrieval through a series of interconnected drop-down menus, where the selection made in one filter dynamically updates the options available in the following. It provides only those options in the drop-down menus that will lead to actual records in the search results. For instance, if 'Trial 2024' is selected in one drop-down menu, the subsequent trait options will be limited to those associated with 'Trial 2024.' This dynamic interac-



EVA Wheat Barley
European Evaluation Network

All Data

Overview

	All Networks	EVA Wheat Barley
Crops	15	3
Accessions	6334	4323
Partner Institutes	90	47
Countries of Operation	33	25
Experiment Locations	116	58
Traits	366	50
Phenotyping Experiments	511	355

Observed Data
323434 PD
Phenotypic Data Points
EVA Wheat Barley, 25-SEP-2024 10:33:21

Accessions
4323 AC
Accessions
EVA Wheat Barley, 25-SEP-2024 10:33:21

Experiments
355 PE
Phenotyping Experiments
EVA Wheat Barley, 25-SEP-2024 10:33:21
13 GE
Genotyping Experiments
EVA Wheat Barley, 25-SEP-2024 10:33:21

Partner Institutes
47 PI
Partner Institutes
EVA Wheat Barley, 25-SEP-2024 10:33:21

Traits
50 TR
Traits
EVA Wheat Barley, 25-SEP-2024 10:33:21

Reference Reports

Figure 2. EURISCO-EVA crop homepage for the EVA Wheat and Barley network showing overview statistics of the network on the left and the shortcut cards leading to reports for metadata and phenotypic data. Additional tabs provide access to pre-filtered data and metadata reports tabs (data as of 25 September 2024).

tion ensures that users are presented with only relevant choices, facilitating a more refined and accurate search.

4. Phenotypic data reports: The interface also includes several types of phenotypic data reports to cater to different reporting needs:

- Default report: Provides a general overview of the data, with each individual data point listed.
- Overview report: Groups data by trait and trial, offering a broader perspective on the data collected.
- Customizable pivot report: Users can create customized pivot reports by selecting a crop and up to five specific traits, along with optional parameters. The report displays the selected traits side-by-side, allowing for a comparative view.

5. Report download options: All reports generated through the interface can be easily downloaded in Excel, CSV, HTML or PDF format. This functionality allows users to further analyze or share their data outside the application.

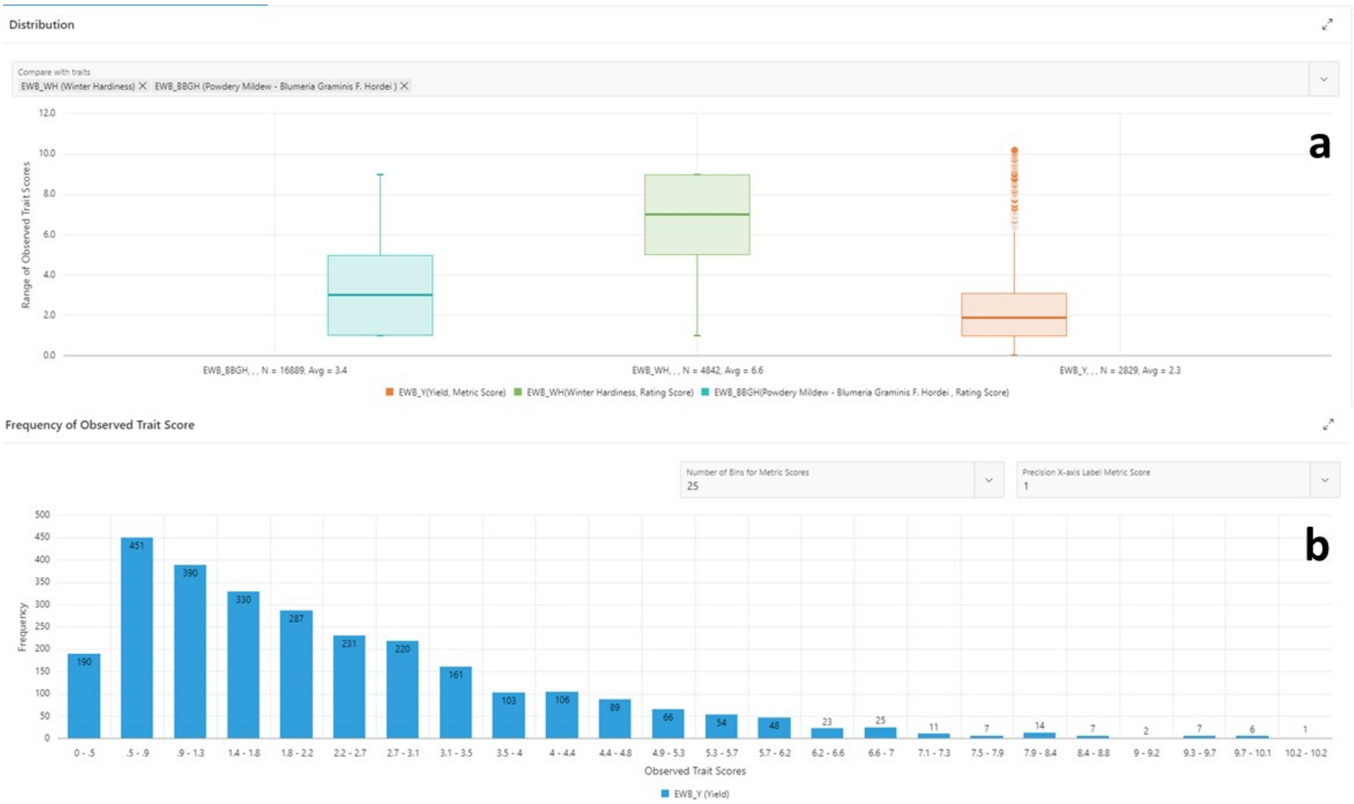
This combination of search methods and reporting tools provides users with a robust mechanism for data retrieval and analysis, ensuring they can access and manipulate the information in ways that best suit their needs.

The chart functionality within the web interface enhances user engagement by offering a flexible and interactive experience (Figure 3). Users can customize charts by selecting data through interconnected drop-down menus, ensuring precision in data selection. Moreover, the chart feature provides access to detailed pages, offering in-depth insights into the viewed data, such as displaying the distribution of data, the frequency of observed values across all (or selected) experiments or comparisons of data collected over different timepoints. Beyond its customizable nature, the chart functionality serves as a gateway to further analysis via the download of associated data as described above. Users can explore detailed reports presenting the total data points per experiment, providing a comprehensive overview of the dataset. Additionally, the chart facilitates the visualization of statistical measures, including mean, variance, median and mode. This multifaceted approach not only empowers users to create personalized visualizations but also supports comprehensive data exploration and analysis through detailed information pages and statistical insights, as can be seen in Figure 3.

Data standardization and upload

In EVA, a big effort was put into the standardization of data collection. The standardization process involves the central maintenance of metadata, i.e. trait definitions, experimental design and passport data. The partners collecting phenotypic data reference these metadata, and the uniform methods and scales of the collected observed phenotypic data make the data easy to understand, analyze and exchange among providers and users.

Generic data collection templates that include all important information for collecting multilocation trial data were developed in order to ease the upload of the data. To reduce the load on background programmes for reading and validating data, and


Compare with traits
EWB_WH (Winter Hardiness) × EWB_BBGH (Powdery Mildew - Blumeria Graminis F. Hordei) ×

Number of Bins for Metric Scores
25

Precision X-axis Label Metric Score
1

Figure 3. Examples of data visualization in the EURISCO-EVA user interface. a) Comparison between distribution of two trait scores using box plot, b) Histogram showing the frequency of scores of a specific trait.

to make the processing faster, a data collection template consisting of two Excel files was created and is available in the documents section of the EVA website (see https://www.ecpgr.org/eva/documents-and-links/evaluation-protocols-and-templates and the current version v1 included as Supplemental data 1 and 2). One data collection template (Supplemental data 2) was designed for collecting and updating the observed evaluation data with minimal trial details and is used by the data providers. The second collection template (Supplemental data 1) is used for creating and updating metadata, including trial details, trait definitions, accession passport data and partner details. This template is centrally completed by the EVA coordinator with input from partners, making the overall data collection simpler, faster and less prone to error. The EURISCO-EVA data templates aim to fulfil the MIAPPE standard in the best possible way while keeping them simple for use by diverse stakeholders. Moreover, most of the accessions that are part of EVA are already documented in EURISCO. To keep the passport data in the two systems consistent, a process was implemented that automatically synchronizes the passport data from EURISCO to the EURISCO-EVA Information System.

The overall phenotypic data upload and management consists of three parts (Figure 4): 1) data file upload: the website features a streamlined four-step file uploader, allowing users to easily upload the phenotypic data using the aforementioned generic data collection template, 2) data processing: the background import programme initiates upon file upload, reads data from the file, validates the information, and subsequently writes it into the designated database tables, 3) data presentation: data is retrieved from these tables and is utilized on the front end to generate reports and charts on the web application (Figures 2 and 3).

Users are only able to upload data for experiments that they are responsible for. If errors are detected during data upload and validation, a message is logged and shared with the user, with a description of the error and an explanation of how to fix it in the data template. Most errors are with formatting or values that are outside the allowed range and the error log enables users to easily identify and fix the issues. Once the processing is successfully finished, an email with the processing log is sent to the user, confirming the successful upload.

Apart from the evaluation data, up to five images per accession can be uploaded to illustrate its appearance and specific characteristics. The images are associated with the accession and thus enrich the passport data available for accessions in EURISCO-EVA. The image uploader allows the user to map the images also to a certain trait or trial, where applicable, which makes the images searchable. Moreover, users can upload additional files to their experiment that contain further trial information. Since the data in these files are not written into the database, they can include various file formats providing for example graphical representation of field layouts or initial statistical analyses. These files

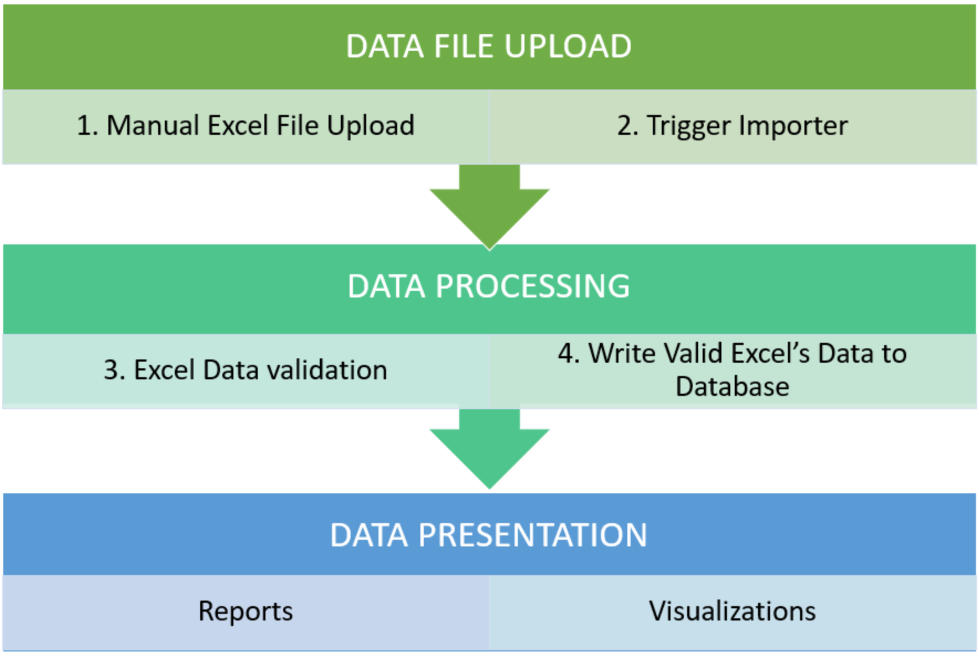


Figure 4. EURISCO-EVA dataflow and capabilities overview.

are included in the trial detail report and are available for download by all users.

Database implementation

The EURISCO-EVA Information System is based on an Oracle relational database management system version 19c. The foundational database schema encompasses 49 tables (see Supplemental Data 3), while the business logic was implemented using PL/SQL, primarily focused on ensuring data quality, enhancing performance, enabling user-specific download capabilities, facilitating reporting tasks, automating data manipulations and uploads, and executing scheduled removal of unnecessary data. The EURISCO-EVA Information System provides a set of functionalities for administrators to maintain the website content. The website is highly scalable to new crop networks and is currently being expanded to host the data of the EVA Legumes network, which started operations in 2024, as well as a demo network for the interested public.

EVA's data model is trial-based, and designed for collaborative, multi-environment evaluations, incorporating data from both public and private partners. Unlike accession-based models like EURISCO, which focus on cataloguing genetic resources, EVA emphasizes ongoing evaluation data collected continuously throughout active trials. Moreover, EVA standardizes trait definitions and measurement scales across all experiments, ensuring that data from one partner is fully compatible and usable by all other partners. This consistency significantly improves data comparability and usability, making it highly valuable for advanced data analysis, including comparative studies, large-scale data mining and breeding decision support. The combination of continuous data collection and standardized traits enables EVA to deliver more actionable, high-quality insights for breeding and research compared to existing solutions.

Compared with other information systems developed for managing data on PGRFA characterization and evaluation such as Germinate (Shaw *et al*, 2017) or Grassroots (Bian *et al*, 2017), EURISCO-EVA is not an open-source project software that can be easily installed and applied to new projects. Instead, EURISCO-EVA is operated by ECPGR as a long-term service to the PGRFA community and is very closely linked to EURISCO as the central European PGR information system.

Outlook

Since its inception, EURISCO-EVA has facilitated data curation and management and enabled the analysis of complex datasets by the existing EVA networks, which have produced several publications (Goritschnig *et al*, 2023; Tripodi *et al*, 2023; Balconi *et al*, 2024). Although EURISCO-EVA has been built as a platform with restricted access to the data, its use of the same background system as EURISCO can easily facilitate the incorporation of phenotypic data in the public database after the end of the data embargo periods. Ensuring the public availability of the generated evaluation data is one of the core values of the EVA networks and will be provided through EURISCO. However, some discussions are still ongoing about how to ensure that useful data is available to the public (e.g. all raw data vs. experiment means, considering also the quality of data from individual trials).

The EURISCO-EVA Information System has a wide range of features for a beginner as well as an advanced user among EVA network partners. A beginner user, who may find manual searches and filters challenging, can easily open reports by applying a pre-defined filter with just one click. A more advanced user can apply searches and filters with several drop-down selection lists to customize their reports. The data collection uploader is self-explanatory, highly intuitive and easy to debug. Besides common features allowing users to view, filter, visualize, email and download data, the web application provides additional features like pivot reporting and data visualization, which take user input to dynamically create custom pivot reports or highly intuitive charts. It is a highly scalable system in which the administrator can easily configure a new network with the existing import programme and template for partners. As mentioned, the EVA Legumes network has recently been added.

In the future, the error management of data processing could be made more robust and user-friendly. Also, an immediate value could be added to the EURISCO-EVA Information System by integrating a data analysis module into it. Moreover, an open-access demo network will be configured so that new potential partners can have the feel of EURISCO-EVA's user interface, background programmes, easy-to-use reports and data visualization features, without accessing restricted data. The EURISCO-EVA Information System is an ongoing initiative that integrates data on PGRFA evaluations from multiple partners and locations. Considering that crop characterization and evaluation data are often scattered in various data sources and publications and lack standardization (Ćwiek Kupczyńsk *et al*, 2016), the EURISCO-EVA Information System provides a user-friendly and versatile environment that enhances data interoperability as well as standardization (by considering uniform traits and methods) of phenotypic data in the framework of the evaluation activities of the EVA initiative.

Conclusions

In this paper, we described the development and implementation of EURISCO-EVA, an information system for PGRFA, which supports the management of metadata and experimental phenotypic data for EVA, with the possibility to provide FAIR public access to data after an embargo through its interoperability with EURISCO. EURISCO-EVA is being maintained by ECPGR and upgrading with additional elements will be possible in the future. EURISCO-EVA provides a gateway for important evaluation data describing genetic resources, adding value to genebank collections and enabling users across the globe to access phenotypic data for genebank accessions through EURISCO.

Acknowledgements

The authors wish to thank all partners of the EVA networks for their feedback on earlier versions of the database and suggestions for improvements and new functionalities. This work was supported by the German Federal Ministry of Food and Agriculture through grant GenRes 2019-2 to ECPGR for the implementation of the EVA networks. The authors are grateful to the two reviewers for their useful comments and suggestions on an earlier version of this manuscript.

Supplemental data

Supplemental data 1: Metadata creation templates
Supplemental data 2: Phenotypic data collection templates for users
Supplemental data 3: Foundational database scheme of EURISCO-EVA

Authors contribution

Suman Kumar was responsible for the initial conception and design. Suman Kumar also wrote the initial draft of the manuscript, with Filippo Guzzon contributing by enhancing and refining the content. Sandra Goritschnig and Stephan Weise reviewed and edited the manuscript, providing critical input to ensure its relevance. All authors read and approved the final manuscript.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

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Analysis of passport data of *Sechium* spp. from the Mexican chayote genebank in Huatusco, Veracruz

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Abstract: Chayote (*Sechium* spp.) (Cucurbitaceae) is a Mesoamerican genus, with Mexico being the primary centre of biodiversity for four species: *Sechium compositum*, *S. chinantlense*, *S. hintonii* and *S. edule*. Mexico also hosts the only chayote genebank in the world, which follows a taxonomic arrangement for its intraspecific variants. Descriptive and multivariate techniques were used to analyze the passport data of the accessions conserved *ex situ* aiming to identify associations and similarity patterns to allow efficient management and origin traceability, stakeholder engagement, consumer preferences, destination, use and conservation practices. Results showed that 23% of the accessions belong to *S. edule* var. *virens levis,* 62.8% come from backyards, and 58.9% of the *S. edule* variants are kept and preserved by women. Interestingly, about 70.8% of the accessions are used for trading, and the rest for self-consumption. Multiple correspondence analyses showed that 27.24% of the first two components variation and the variables with the greatest phenotypic contribution were fruit flavour (sweet and bitter), condition of the populations (forest, ruderal-wild, orchard), fruit colour (yellow, light green), small size and flattened shape. The morphological boundaries of *S. edule*, *S. compositum* and *S. chinantlense* variants are defined by the fruit morphology. These findings from passport data analysis support the development of strategies for replacement, regeneration, distinction, genetic improvement, conservation and bioprospective studies.

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Introduction

Plant genetic resources for food and agriculture are the basis of human nutrition, industrial inputs and pharmacologically active ingredients. Cereals, fruits, roots and vegetables contribute significantly to agrobiodiversity (Bellon *et al*, 2009). The *ex situ* conservation of underutilized landraces and wild relatives has become a relevant source of unexplored genes, enriching germplasm banks and their use in breeding. Mexico is a megadiverse country, and rural communities manage a wide range of domesticated and semi-domesticated biological variants, cultivated and wild relatives which significantly increase agrobiodiversity (Casas and Vallejo, 2019). Field collections of species with recalcitrant seeds, such as chayotes (genus *Sechium* P. Br.), are among the strategies for *ex situ* conservation of agrobiodiversity. Chayote is an increasingly important vegetable in international markets, and morphotypes of the

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Mexican clade have gradually been collected (Barrera-Guzmán *et al*, 2021a).

In Mexico, *Sechium compositum* (Donn. Sm.) C. Jeffrey, *S. chinantlense* Lira & F. Chiang, *S. hintonii* (Paul G. Wilson) C. Jeffrey and *S. edule* (Jacq.) Sw have been recorded as endemic species. Among these, only *S. edule* is edible since the other three species have bitter-tasting fruits. *S. edule*, particularly the smooth green var. *virens levis* variant, is distributed on all continents (Cadena-Iñiguez, 2005). Its success as a vegetable has transcended local markets, becoming an export product (Cadena-Iñiguez and Arévalo-Galarza, 2011). Different studies highlight *S. edule* as a species with notable intraspecific diversity, contributing to the feeding and rural local economy (Aguirre-Medina *et al*, 2021). However, most published research only mentions *S. edule* without specifying the biological variant. For example, Dire *et al* (2003), Setzer and Setzer (2003), Ordoñez *et al* (2006), Loizzo *et al* (2016), Vieira *et al* (2019), among others, do not specify the variants studied, making it challenging to reproduce their findings. Lira *et al* (1999) performed a grouping analysis of edible morphotypes of *S. edule* and another for a wild type, without distinguishing or specifying the variation. This makes conservation actions difficult since morphotypes must be identified by some taxonomic method to be included in genebanks. In this case, the popular common name is not appropriate.

Morphological, anatomical, biochemical and genetic studies, carried out by various authors (Donato *et al*, 1994; Cadena-Iñiguez, 2005; Cadena-Iñiguez and Arévalo-Galarza, 2011; Iñiguez *et al*, 2011; Avendaño-Arrazate *et al*, 2012; Machida-Hirano *et al*, 2015; Barrera-Guzmán *et al*, 2021a,b; Iñiguez-Luna *et al*, 2021), have shown that there are stability, heritability and uniformity traits that allow the distinction of *S. edule* variants, which facilitate their conservation, management and research. These traits confer different and desirable characteristics for its use as food (Cadena-Iñiguez *et al*, 2013a; Aguiñiga-Sánchez *et al*, 2015, 2017; Salazar-Aguilar *et al*, 2017). Genebanks are crucial to avoiding diversity loss and maintaining this agrobiodiversity. In 2005, the National Germplasm Bank for *Sechium edule* (BANGESe) was founded to preserve endemic species and biological variants. To achieve this, fruits were collected from 12 Mexican states, as well as from Guatemala and Costa Rica. Currently, the BANGESe safeguards more than 300 accessions in a field collection since *Sechium* seeds are recalcitrant and do not respond to traditional conservation methods (Ramírez-Rodas *et al*, 2021).

The accessions are conserved in a taxonomic arrangement following the principles by (Stace, 1986) and adapted for *S. edule*. It integrates morphotypes as varietal groups that possess stable, heritable characteristics and fertile offspring. These varietal groups are *albus minor, a. levis, a. dulcis* and *a. spinosum* for plants with yellow, smooth and prickly fruits. For fruits with dark green epidermis with and without thorns are *nigrum minor, n. conus, n. levis, n. xalapensis, n. spinosum* and *n. maxima*. The morphotype *virens levis*, the most widely distributed in the world, reports light green fruit without spines, while the only representative with bitter fruits of *S. edule* is the *amarus sylvestris* morphotype. The above taxonomic nomenclature has facilitated the handling, distribution and distinction of accessions, allowing research, bioprospecting, breeding and preservation activities.

As in many germplasm collections, BANGESe accessions come from direct collection from the areas of origin, distribution and domestication, as well as from donations by rural inhabitants who have locally safeguarded agrobiodiversity for a particular interest. This raises several important questions related to the management, design and regeneration strategies of these resources. For example: Which of the *Sechium* varietal groups have the broadest geographical distribution? Which biological variants (morphotypes) are the most important in terms of consumer preferences and what characteristics drive this? What is the origin of the accessions, and what implications does this have for their conservation? Which stakeholders are most engaged with conservation efforts? What are the primary uses of the fruits? How does the distribution of varietal groups vary with altitude? Finally, what are the morphological traits of the fruits that facilitate their visual distinction?

Genebanks are long-term conservation centres of genetic resources, especially to preserve agrobiodiversity. However, without thorough characterization and documentation of the accessions, making informed decisions about conservation, research, genetic improvement and potential use is challenging (Weise *et al*, 2020). Since *Sechium* is a cross-pollinated plant, asexual methods of multiplication are used to preserve the genetic identity of the accessions. The most successful are grafting, rooting of cuttings, and in vitro multiplication (Figure 1).

Materials and methods

Location of the genebank and passport data

The National Germplasm Bank of *Sechium edule* (BANGESe) is located in Huatusco, Veracruz, Mexico (19° 08’ 48” N, and 97° 57’ 00” W). The vegetation type is mountain cloud forest (altitude of 1,340 masl), with a mean annual temperature of 19–22°C, 85–90% relative humidity, and 2,250mm mean annual precipitation. The soils are vitric luvisol, rich in organic matter, low calcium and high iron, manganese and zinc nutrients, with moderate fertility, coarse texture and volcanic glass fragments (pH 4.3–6.5). The current area of the genebank covers 3ha. Because chayote is a climbing plant, accessions are kept in a metal net-like support structure at a 2.2m height. The accessions are introduced into the genebank as ±20cm plants, obtained by direct sampling, donation or purchase from rural inhabitants. *Sechium* plants are cross-pollinated. To maintain the genetic identity of each accession, asexual

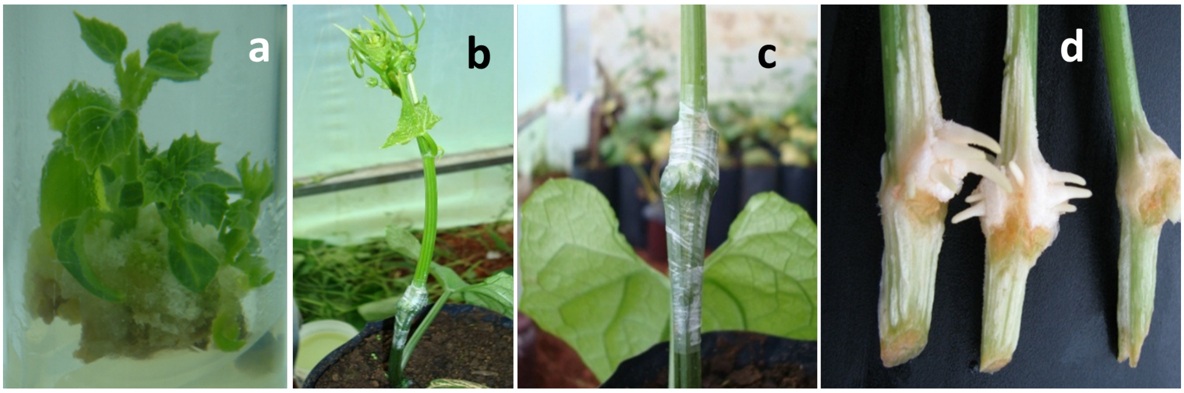

Figure 1. Methods of regeneration and asexual multiplication of *Sechium* spp. accessions. a) *In vitro* multiplication, b-c) grafting and c) callus emission, training sites and emission of roots from the use of regulators of growth.

multiplication techniques such as grafting, rooting of three-node cuttings, and in vitro multiplication are employed.

Statistical analysis

The passport data in BANGESe include georeferenced and taxonomic data, origin, morphological characteristics of fruits and seeds, biological status and ethnobotanical information, following the descriptor guide for *S. edule* varieties (Cadena-Iñiguez *et al*, 2017). Passport details can be found at Bangermex (https://bangermex.snics.gob.mx/bancos). The information was analyzed with the Rstudio software (R Core Team, 2023). Out of a total of 309 accessions, only 231 were analyzed, since 78 were excluded being genetic segregants from the hybridization programme. Graphic analysis was performed with tidyverse (Wickham *et al*, 2019) to assess the proportion of varietal complexes regarding their origin, type of establishment (orchard, backyard, ruderal) and owner/donor. Additionally, the central tendency and dispersion statistics of the altitude variable were evaluated to know any pattern related to the distribution ranges at which *Sechium* varietal complexes develop. Qualitative variables were studied by multiple correspondence analysis (MCA) with FactoMineR (Lê *et al*, 2008) and factoextra (Kassambara and Mundt, 2020). To calculate the eigenvectors, eigenvalues and the graph of each variable's contribution to the components, the same statistics used for the MCA were employed.

Results

Descriptive analysis

BANGESe contains 231 accessions representing the intraspecific variability of *S. edule* in 12 varietal complexes: *albus dulcis*, *albus levis*, *albus minor*, *albus spinosum*, *amarus sylvestris*, *nigrum conus*, *nigrum levis*, *nigrum maxima*, *nigrum minor*, *nigrum spinosum*, *nigrum xalapensis* and *virens levis*. Also, there are four accessions of *S. chinantlense*, 12 of *S. compositum* and two of *S. hintonii* (the latter was not analyzed). The *virens levis*, *n. spinosum* and *n. xalapensis* varietal complexes are the most represented in the collection (Figure 2a). The major diversity of *S. edule* in Mexico is found in the state of Veracruz, with 110 accessions (47.6% of the total) (Figure 2b). Of the 231 accessions, 62.8% were collected from backyards and 23.3% from commercial orchards, while *S. compositum*, *S. chinantlense* and *S. edule* var. *amarus sylvestris* came from ruderal and forest areas (Figure 2c). Some accessions of *virens levis*, *n. spinosum* and *n. xalapensis* were from Costa Rica (CR) and Guatemala (Guat) (Figure 3).

It is well known that women play a key role in vegetable cultivation and plant breeding in domestic orchards. Our data showed that women managed 58.9% of the *S. edule* accessions, all of which are edible. Wild accessions are outside the interest of men and women; however, their conservation in genebanks is important due to their phytochemical potential for pharmacological use and as sources of genes for genetic improvement in the face of new challenges. The varietal complexes *virens levis, nigrum xalapensis, n. spinosum, n. levis* and *albus dulcis* were identified as man-enterprise, due to their large-scale commercialization. The 70.8% of the varietal complexes are cultivated for commercial purposes, while 29.2% are used for self-consumption, mainly focusing on fruit. This is especially relevant for the lesser-known variants, highlighting their potential to open new markets. The *virens* and *nigrum* groups are mainly used for commercialization, while *albus* is used for self-consumption. Bitter-flavoured varieties are generally not used by rural stakeholders (Figure 4).

Sechium sp. can be found at altitudes from 1,200–3,376m, due to its plasticity, which allows it to adapt to conditions different from its original habitat in mesophyll forests (Cadena-Iñiguez *et al*, 2008). The highest average altitude for *nigrum spinosum* was 1,993m. Outliers' presence affects some central tendency measures such as the mean, and some populations of the varietal complexes appear far from their optimal ranges for each specific variety.

Multiple correspondence analysis (MCA)

For this analysis, the phenotypic variables of fruits were included (colour, presence/absence of thorns, size,

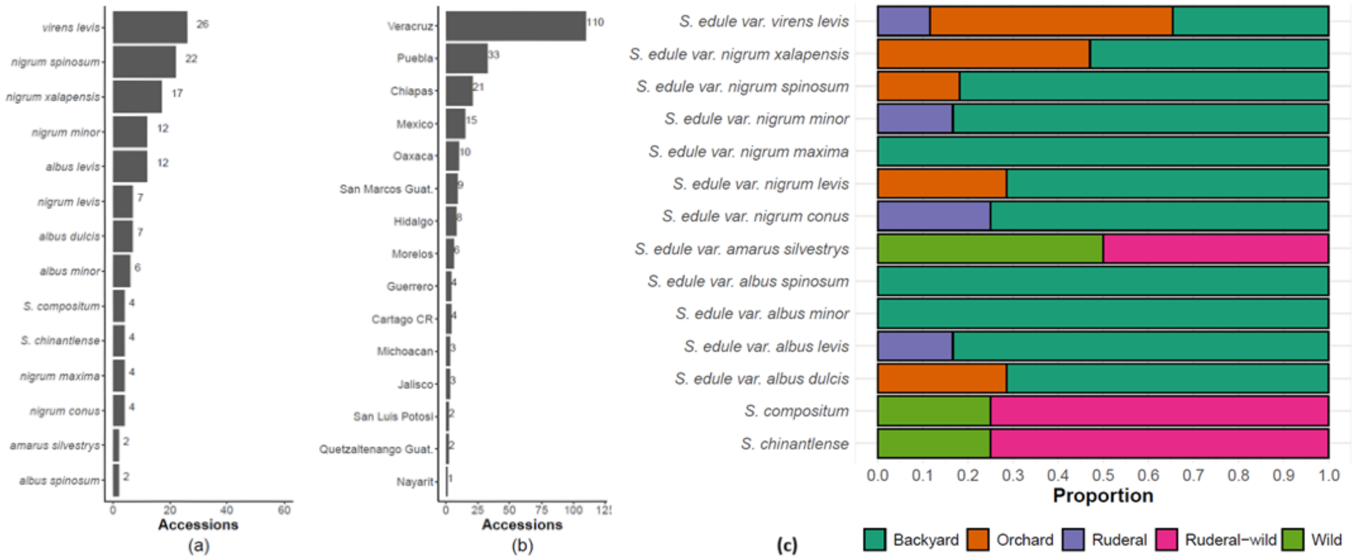

Figure 2. Composition of the *Sechium* collection at BANGESe. (a) Number of accessions per varietal group of *S. edule*, *S. compositum* and *S. chinantlense*; (b) Geographical origin of the accessions; (c) Sampling locations.

flavour and shape). The first two principal components explained 27.24% of the total obtained variation. Table 1 shows the eigenvalues and eigenvectors, respectively. For principal components 1 and 2 (PC1 and PC2), the variables contributing the most were fruit flavour, populations condition (forest, ruderal-wild and orchard), fruit colour (yellow and light green), small fruit size and flattened shape.

The distribution of the accessions depended on the environmental conditions. Accessions of *S. compositum*, *S. chinantlense* and *S. edule* var. *amarus sylvestris* were distributed in wild and ruderal-wild forms. The *virens levis* complex was primarily found in orchard conditions and is the most commercially important variety of S. edule. The yellow-fruited varietal complexes were generally found in backyards. Ownership patterns indicate that the *virens levis* varietal group was more often associated with male farmers, while female tended to maintain the *albus* and *nigrum* groups.

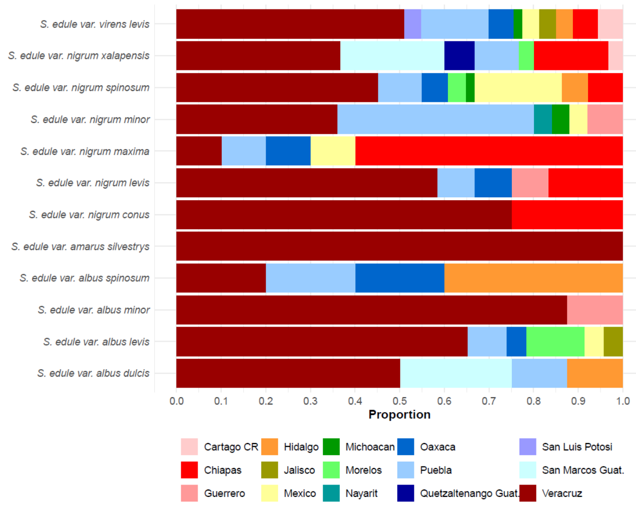

Figure 3. Proportion and distribution of *Sechium edule* varietal groups in the BANGESe collection according to place of origin of *Sechium* germplasm. CR, Costa Rica; Guat, Guatemala.

Regarding fruit colour, most accessions exhibited various shades of green, except for the *albus* group, which had yellow fruits (Figure 5a). Figure 5b shows that all accessions of *amarus sylvestris* (AW), *nigrum spinosum* (NS) and *albus spinosum* (AS) had thorns on their fruits. This is relevant, considering that the wild ancestor *S. edule* (wild type) is dark green, bitter and densely spiny, suggesting that consumer preference is for smooth fruits and neutral or sweet flavour.

The fruit size of the chayote is highly variable, ranging from very large (15–22cm), medium (6–10cm), and small (3–4cm) (Figure 6a). Regarding flavour, the ellipses show a very noticeable separation, revealing that the fruits of *S. chinantlense*, *S. compositum* and *amarus sylvestris* are bitter, a trait attributed to their tetracyclic triterpenes content, mainly cucurbitacins (Aguiñiga-Sánchez *et al*, 2015, 2017; Salazar-Aguilar *et al*, 2017). Fruits of the *albus* group have a slightly sweet flavour (7.6–8.1°Brix), while *nigrum* (4.9–6.4°Brix) and *virens* groups have a neutral flavour (5.1°Brix) (Figure 6b) (Cadena-Iñiguez *et al*, 2007; Cadena-Iñiguez and Arévalo-Galarza, 2011).

Cucurbitacins are secondary metabolites of triterpene origin that give a bitter taste to plants (root, guides, leaves, and fruits) and have been recorded in all *Sechium* species in the BANGESe, although with notable differences in concentration and type of compound (aglycones and glycosides). For instance, the *albus* group contains one-hundredth of the triterpenes levels found in wild species, and ten times less than nigrum (Uriostegui, 2014; Iñiguez-Luna *et al*, 2021).

Fruit shape is highly variable and it is difficult to establish a clear grouping pattern (Figure 7); however, the commercially preferred shape is pyriform, which is the shape of *virens levis* and is the basis of the international standard Codex-Stan-83-993 (pyriform

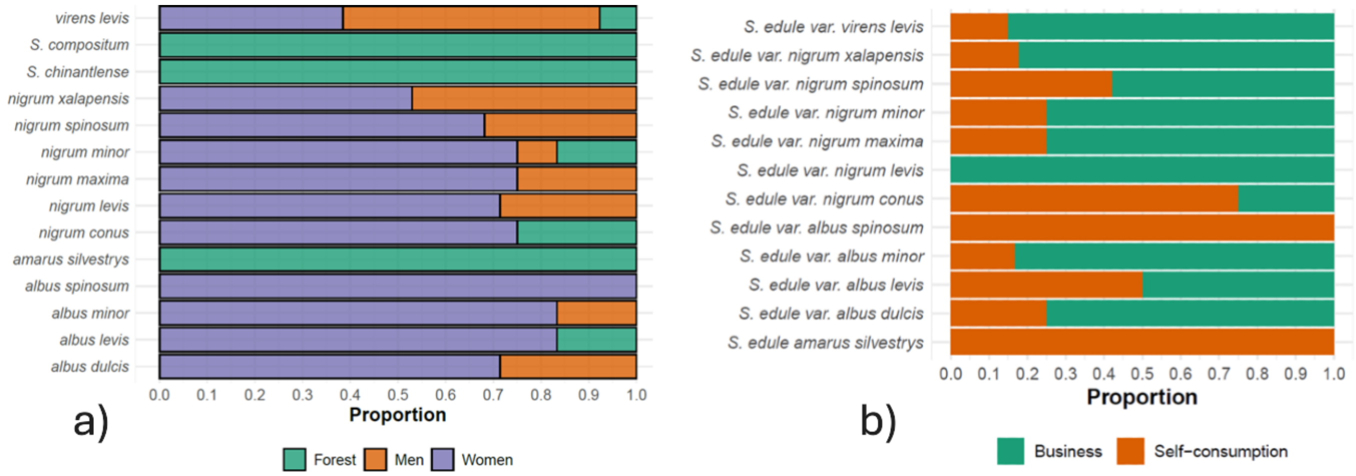

Figure 4. Relationship between stakeholders and conservation of *Sechium edule*, *S. chinantlense* and *S. compositum* of the *Sechium* germplasm collection at BANGESe; a) the conservation methods/users and b) the use of the accessions.

Table 1. Eigenvalues and variance are explained for the first ten components of the MCA. PC, principal component.

Component	Eigenvalue	Variance (%)	Cumulative variance (%)
PC 1	0.49	15.50	15.50
PC 2	0.42	13.48	28.98
PC 3	0.30	9.48	38.46
PC 4	0.24	7.79	46.24
PC 5	0.20	6.49	52.74
PC 6	0.19	6.16	58.90
PC 7	0.18	5.82	64.72
PC 8	0.16	5.20	69.92
PC 9	0.15	4.82	74.73
PC 10	0.15	4.70	79.44

fruits, light green colour, smooth without thorns and grooves, 12–15cm long, 8–10cm equatorial width, 270–310g, neutral flavour, and free of pathogens). The pyriform shape may be a derivation of continued selection by cultivars, as the *S. edule* fruits of the wild type (ancestor) are small and ovate (Figure 8).

Morphological boundaries

Establishing morphological limits in intraspecific complexes is relatively difficult, since it requires to determine whether distinctive traits are stable and heritable. Variation in plants may be continuous (clinal) or discontinuous, consisting of distinct morphotypes or races (Styles, 1986). In *Sechium*, variation is clinal since it shows a gradient that allows any character to take values between two extremes (Cadena-Iñiguez, 2005). Environmental factors, including numerous physical, chemical and biological sub-variables, induce this variation by exerting extra pressure on the differentiation process, enabling organisms to express a sufficiently satisfactory plasticity response for survival. For example, in *S. edule* and *S. compositum*, fruit shape and size vary according to altitude, with accessions from lower elevations (60m) producing round and small fruits, while those from higher elevations (200m) produce medium pyriform fruits in evergreen forests. Figure 9 shows the morphological limits based on fruit shape, which helps to distinguish the varietal complexes of *S. edule*, *S. chinantlense* and *S. compositum*, based on the colour of the epidermis, spines, shape and size.

Discussion

The preference in the consumption of chayote varietal groups is based on the physical and chemical characteristics of its fruits. In Mexico, the most present in the markets are *virens levis*, *nigrum xalapensis* and *nigrum spinosum*, the latter recently introduced in the United States. For these markets, fruits are harvested at horticultural maturity (Aung *et al*, 1990) and have a watery consistency, neutral flavour and little fibre content. In regional markets, fruit with higher starch content in the endocarp, such as *albus*, or physiologically mature green fruits, are preferred. This promotes unconscious conservation actions in backyards by women, who determine the type of chayote to be used in the diet. Their decision to either conserve a single morphotype or diversify indirectly leads to reproductive isolation and inbreeding selection, which helps to fix characters and make them stable and heritable or may induce spontaneous crosses (Cadena-Iñiguez *et al*, 2010).

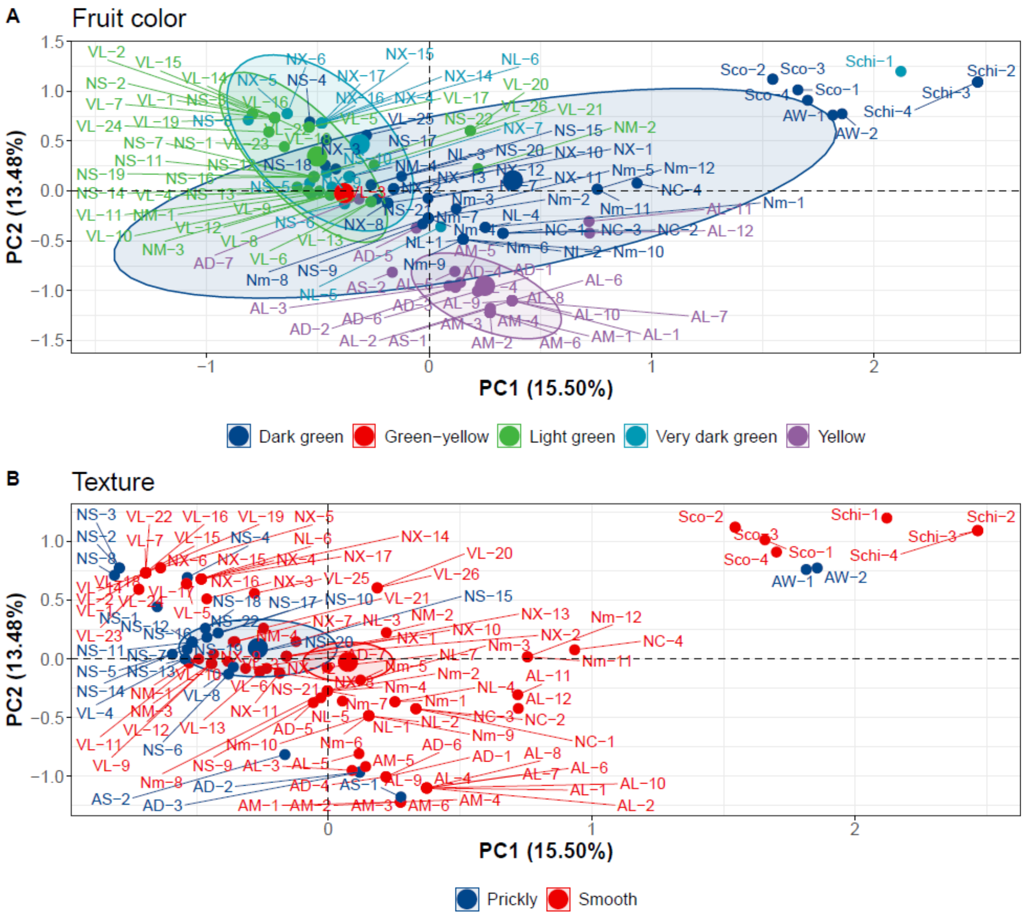

Figure 5. Multiple correspondence analysis for A) Fruit colour, and B) Fruit texture variables on *Sechium* germplasm. AL, *albus levis*; AS, *albus spinosum*; AW, *S. edule* var. *amarus sylvestris*; NC, *nigrum conus*; NM, *nigrum maxima*; Nm, *nigrum minor*; NS, *nigrum spinosum*; NX, *nigrum xalapensis*; Sci, *S. chinantlense*; Sco, *S. compositum*; VL, *virens levis*. PC, principal component.

Women play a key role in the conservation of plant genetic resources mainly through the management of family orchards, where these resources are used for food and sometimes for local sale. Local marketing or exchange of backyard surpluses allows genetic diversity to flow at the regional level (Watson and Eyzaguirre, 2002). When phytogenetic resources are used for human consumption, strong selection pressures arise. For chayote, the preference tends to converge on green fruits with neutral or sweet flavour and low fibre, although preferences for fruits with starchy consistency (*albus* group) have also been identified (Cadena-Iñiguez *et al*, 2010).

The first wild populations of *S. edule* evolved in mesophyll forests, producing dark green, spiny and bitter fruits (Lira, 1996). This type of vegetation is conducive to intraspecific variation in *S. edule* (Iñiguez *et al*, 2011). Mesophilic forests, distributed across the highlands of the Sierra Madre Oriental, Sierra Norte de Chiapas and Sierra Madre del Sur, range from 600–3,100m in altitude, with annual precipitation of 1,000-3,000mm, and temperatures ranging between 12–23°C (Ruiz-Jiménez *et al*, 2012).

Along with natural and artificial selections, migratory routes also cause variations in fruit colour and flavour. When wild populations of *S. edule* spread to low sub-evergreen forests, greater irradiance led to a change in chlorophyll content (Iñiguez *et al*, 2011), giving rise to some varietal complexes such as *virens levis* and those of the *nigrum* group. In the high plateaus and high valleys, it is common to find thorny green varietal complexes. In medium evergreen and low deciduous forest vegetation types, leaves and fruits began to change in colour and shape, later giving rise to varietal complexes of the *albus* group, where the fruits have low chlorophyll and cucurbitacin content but high carotenoid and ascorbic acid content (Iñiguez *et al*, 2011), presumably for photoprotection, since these fruits have high stomata density (Ramírez-Rodas *et al*, 2021). Also, the derivation of yellow fruits is strongly influenced by selection pressures. Valenta *et al* (2018) indicate that fruit colour plays an important role in seed dispersal and can be considered an evolutionary signal in plants. A contrast between leaf and fruit colour is important for zoochory success. In this regard, Iñiguez *et al* (2011) mention that yellow chayotes are considered the most evolved in terms of their environmental adaptation.

MCA of the fruit size shows that this variable tends to be dynamic. Wild populations of *S. edule*, *S. chinantlense* and *S. compositum* have relatively small fruits (Figure 9) and bitter taste. The latter is related to bi-1 and Bt-1 genes, which confer bitter taste to leaves and

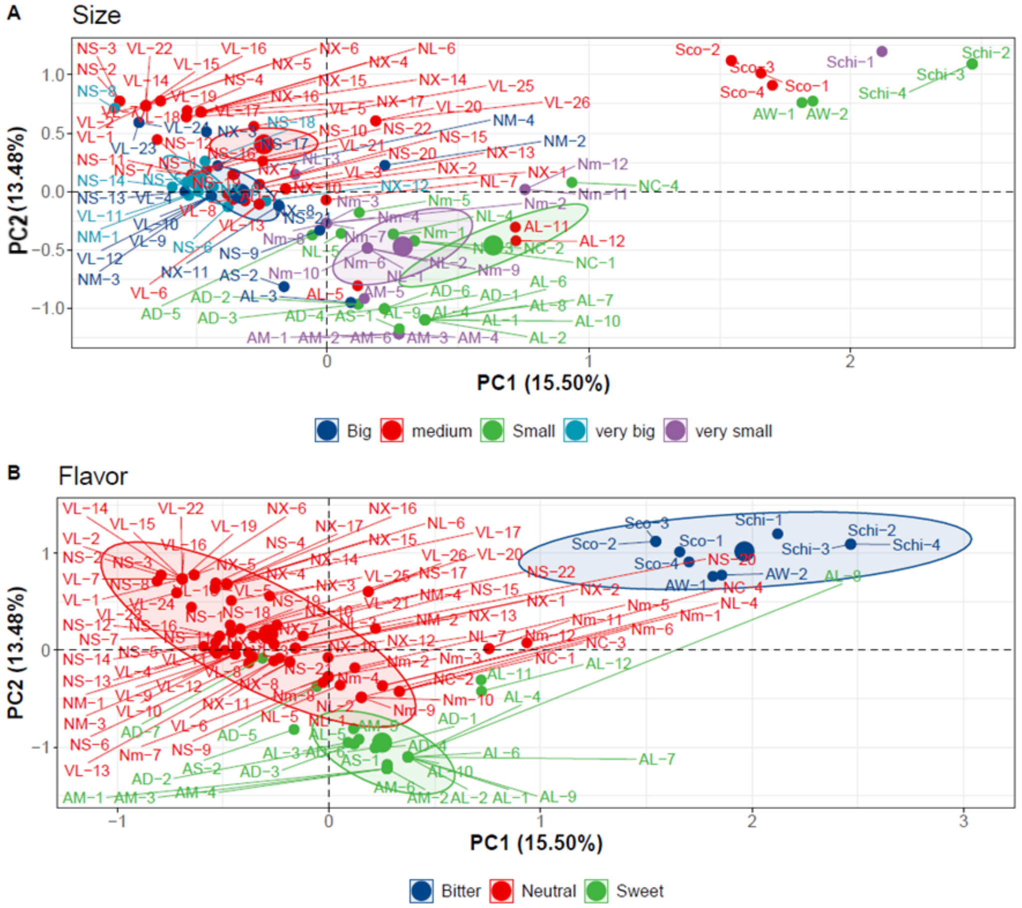

Figure 6. Multiple correspondence analysis for the variables A) Size, and B) Flavour of fruit on *Sechium* germplasm. Sco, *S. compositum*; Sci, *S. chinantlense*; AW, *S. edule* var. *amarus sylvestris*; VL, *virens levis*; NM, *nigrum maxima*; Nm, *nigrum minor*; NX, *nigrum xalapensis*; NC, *nigrum conus*; NS, *nigrum spinosum*; AL, *albus levis*; AS, *albus spinosum*, PC, principal component.

fruits (Valenta *et al*, 2018). Subsequently, with recurrent selection, this characteristic is reduced. In *Sechium* spp., the bitter taste is influenced by the concentration of cucurbitacins, higher in wild populations, and has applications in pharmacology particularly in cancer treatment (Cadena-Iñiguez *et al*, 2013b; Rosado-Pérez *et al*, 2019).

Numerous examples illustrate the role of rural women in conserving plant diversity in home gardens for family food, which in turn supports broader biodiversity. In Indonesia, Elfrida *et al* (2020) recorded the conservation of 39 fruit plant species of 23 genera and 17 botanical families by women. Other studies such as Tefera and Kim (2019) demonstrated the diversity of medicinal plants conserved by rural families in Ethiopia (52 families and 96 genera). Rural women are critical to conserving local knowledge, encompassing beliefs, medicine, food and economic livelihood. Conservation behaviour often arises from culture rather than formal education. Ondiba and Matsui (2021) mention that, from a sample of rural women surveyed, 98% expressed motivation to obtain economic income through acts of conservation, and 88% expressed a commitment to environmental conservation.

George and Christopher (2020) recorded the high species diversity in home orchards in Kerala, India; others, such as Zhang *et al* (2020) documented the plants and their local function, highlighting that altitude could be the most important variable that determines the composition of home gardens in China. Galluzzi *et al* (2010) reported the importance of home orchards in safeguarding agrobiodiversity, which reflects a complex structure.

According to the passport data, the variability of *Sechium* accessions in BANGESe is mainly attributed to the actions of rural women in home gardens or backyards, despite conservation not being their conscious objective. However, and for the reasons documented in the passport data, aspects such as self-consumption, local trade, medicinal and ancestry uses, have fostered a wide agrobiodiversity, mainly determined by these plants' value as food.

An important aspect that interacts with conservation, agrobiodiversity, bioprospective research, new applications, industry, rural players, home orchards and genebanks, are international treaties. The Nagoya Protocol (CBD, 2011) addresses several of these aspects and seeks, as far as possible, a fair and equitable distribution of benefits to the stakeholders that have safeguarded agrobiodiversity or have enriched collections in genebanks. In this regard, the analysis of passport data is a useful tool for identifying potential beneficiaries for bioprospective research and products over the

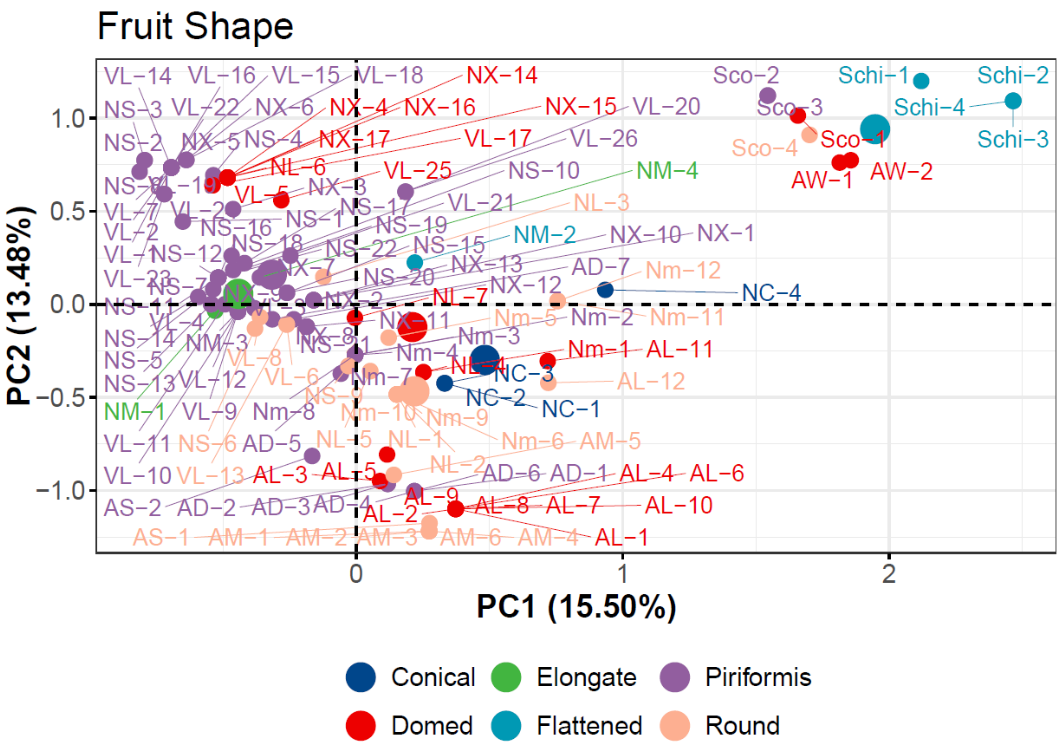

Figure 7. Multiple correspondence analysis for the fruit shape variable of *Sechium* germplasm in the BANGESe collection. Sco, *S. compositum*, Sci, *S. chinantlense*; AW, *S. edule* var. *amarus sylvestris*; VL, *virens levis*; NM, *nigrum maxima*; Nm, *nigrum minor*; NX, *nigrum xalapensis*; NC, *nigrum conus*; NS, *nigrum spinosum*; AL, *albus levis*; AS, *albus spinosum*, PC, principal component.

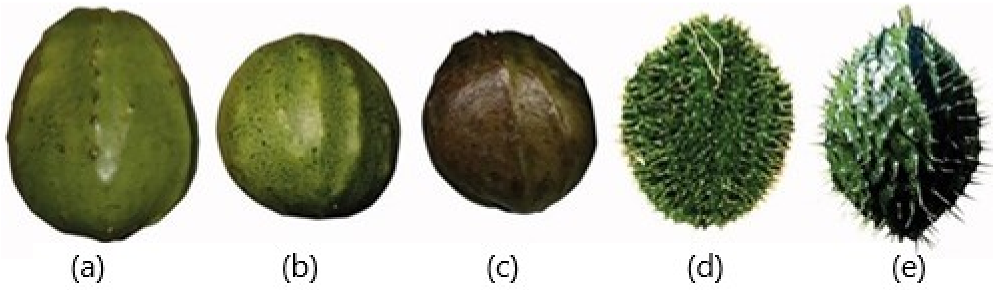

Figure 8. Colour, shape and fruit size variation in *Sechium compositum* (a and b, 6.0–8.0cm), *S. chinantlense* (c, 5.0–6.0cm), *S. edule* (wild type: d, 5.0–6.0cm) and *S. edule* var. *amarus sylvestris* (e, 6.5–7.0cm).

medium or long term. Varshney *et al* (2020) proposed a 5G approach to conserved agrobiodiversity: Genome of each crop species, Genomically and agronomically characterized germplasm, Gene function identification, Genomic breeding methodologies, and finally Gene editing.

This approach is a desirable strategy for germplasm collections; however, it does not consider the participation of rural stakeholders, whose ancestral knowledge and practices have helped maintain high agrobiodiversity. Therefore, analyzing passport information is key to designing strategies for replacing and regenerating genotypes in *ex situ* collections, as well as for designing shared conservation models with rural stakeholders. Such models could include morpho-anatomical, phytochemical, genomic and agronomic characterization lines, as well as bioprospective studies to explore new applications and benefits. These efforts could also support descriptor guides for legal registration of new plant varieties or facilitate compliance with international treaties and material transfer agreements.

Wild populations with small, dark green fruits, thorns and bitter taste were the promoters of the morphological diversity that is currently known of *S. edule*. However, there is also evidence of introgression and hybridization with the species *S. compositum* and *S. chinantlense* (Barrera-Guzmán *et al*, 2021a). Conserving this valuable resource begins with evaluating and characterizing genetic variability through morphological and molecular markers, as well as establishing evolutionary relationships among wild, cultivated populations and related species (Cadena-Iñiguez *et al*, 2007). Ecology studies in conjunction with ecological niche models also provide information on species adaptability, highlighting

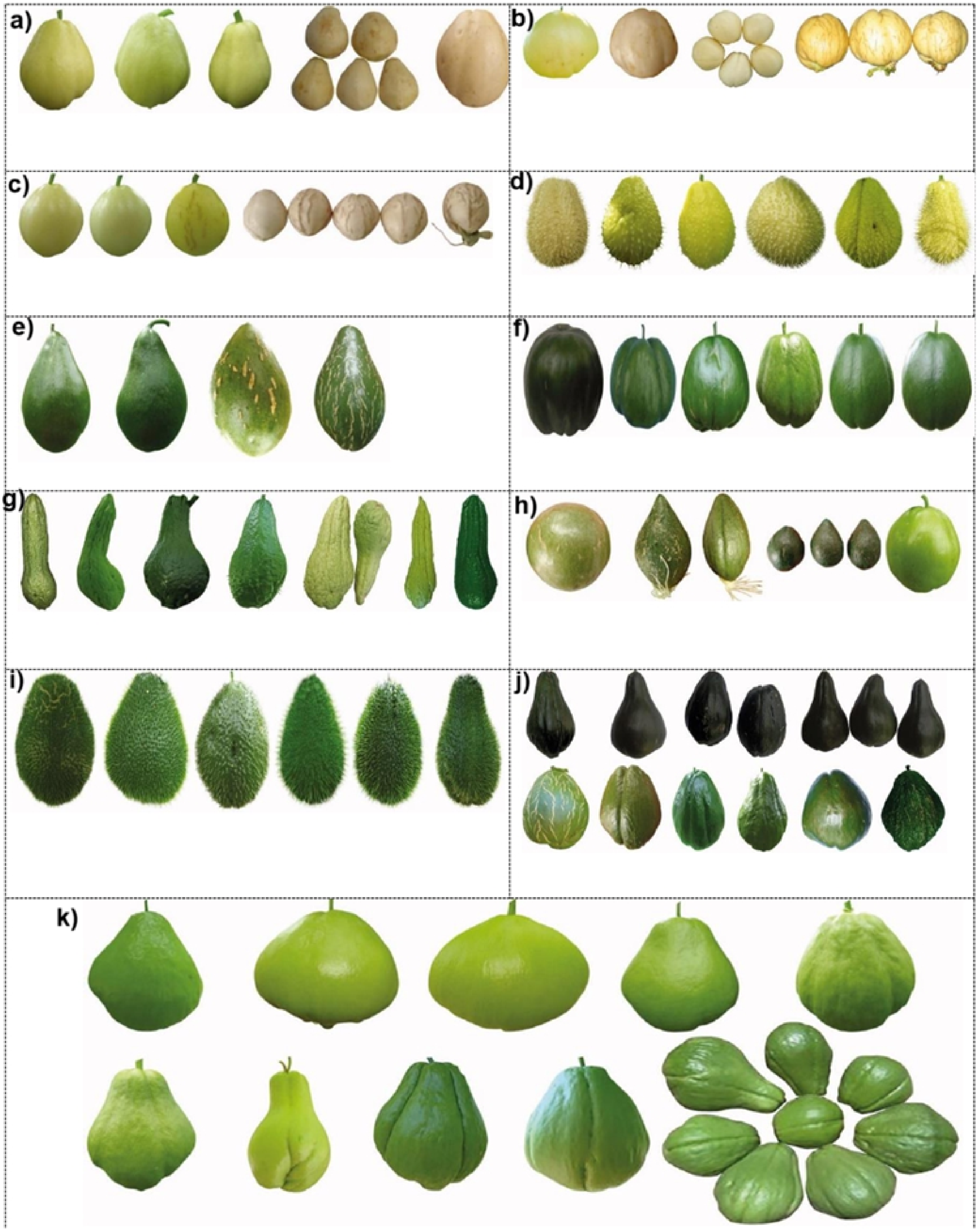

Figure 9. Colour, shape and fruit size variation in varietal complex of *Sechium edule*; a) *albus dulcis* (6.2–7.0cm), b) *albus levis* (6.0–7.0cm), c) *albus minor* (3.0–3.5cm), d) *albus spinosum* (13–16cm), e) *nigrum conus* (5.5–7.5cm), f) *nigrum levis* (5.7–7.0cm), g) *nigrum maxima* (16–22cm), h) *nigrum minor* (3.0–3.5cm), i) *nigrum spinosum* (10–16.5cm), j) *nigrum xalapensis* (15–17.5cm) and k) *virens levis* (13–16cm).

potential areas for conservation and zones threatened by climate change.

The study of biodiversity is also an incentive for participatory genetic improvement programmes of chayote, fostering collaboration and knowledge exchange between researchers and farmers. This encourages the continuous planting of native varieties or populations in traditional plots, and economic support and public policies are essential to maximize these efforts. Additionally, awareness campaigns are needed to inform the population about the importance of chayote as a plant genetic resource (Aguiñiga-Sánchez *et al*, 2017).

It is important to highlight that cytological information is missing or scarce in many accessions both in chromosome number and ploidy level as well as nuclear amount. The chromosomal level variegation such as translocation or inversion could explain species incompatibility (Olvera-Vazquez *et al*, 2019).

Conclusion

The morphological diversity of *S. edule* lies mainly in fruit characters, where traits such as bitterness, the presence of thorns and a dark green colour are representative of wild populations. Geographical and cultural richness also influence crop diversification, and the mountainous areas of Veracruz offer the ideal habitat for growth and development of new varieties. Women play a vital role in the conservation of native chayote populations through backyard cultivation. *S. chinantlense* and *S. compositum* are species related to *S. edule*, and from which important advances are being made in the production of cucurbitacins for the pharmaceutical sector.

Data availability statement

To facilitate access to the data from our chayote germplasm collection, information on how interested researchers can get full access is provided below. The data is hosted in our online database, which is accessible upon request. Researchers interested in accessing the full dataset can do so by contacting the corresponding author and providing a brief description of the intended use of the data. In addition, access requests will be reviewed to ensure proper use in accordance with established ethical guidelines for the conservation and research of plant genetic resources. Our goal is to support collaborative research efforts and encourage studies that expand the knowledge and utilization of this important collection of germplasm.

Authors contribution

Jorge Cadena-Iñiguez y Luis A. Barrera-Guzmán wrote the article and performed the statistical analysis of the database; Víctor M. Cisneros-Solano, the curator of the genebank, provided the information on the morphological aspects of the collection; Carlos H. Avendaño-Arrazate, Ma. de Lourdes C. Arévalo-Galarza, Kazuo N. Watanabe and Jorge D. Cadena-Zamudio, who have been developing the database over the years, also contributed to the discussion of this research.

Conflict of interest statement

The authors have no conflicts of interest to report.

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A case study on lentil to demonstrate the value of using historic data stored in genebanks to guide the selection of resources for research and development projects

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Abstract: Plant genetic resources are essential for sustainable agriculture and a secure and stable global food supply. One of the most important pulses and an integral part of a healthy diet is lentil (*Lens culinaris* Medik.). The National Centre for Plant Genetic Resources of Ukraine (NCPGRU), based in Kharkiv, manages a lentil collection of 1,140 accessions. In 2019, the first exchange of 37 lentil accessions between NCPGRU and a French research team took place. This exchange was accompanied by the transfer of phenotyping data for multiple traits. Considering that data collected in different environments provide important information on trait stability, the lentil accessions were phenotyped under new conditions through field research. This research allowed a comparative analysis of agroclimatic conditions for lentil cultivation in Ukraine (Kharkiv region) and France (Bourgogne-Franche-Comté region). The possibility of using genebank information to guide plant material selection for research and development projects was assessed. As a result, six lentil genotypes that performed well in different environments were selected. This allowed the identification of genotypes with the highest yield potential: UD0600086, UD0600145, UD0600437, UD0600530, UD0600550 and UD0600638. Genotypes recommended for use in breeding to produce high-yielding, relatively stable lentil varieties were successfully selected in the application case of this study, confirming that the information in the trait database of the NCPGRU Genebank is an important resource for predicting the characteristics of lentil accessions. Of the eight accessions selected by GGE biplot analysis using field research, five were predicted to be more promising by previous genebank data.

Keywords: lentil (*Lens culinaris* Medik), pulses, genetic resources, genebank trait database, breeding material

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Introduction

Sustainable agriculture and global food security depend on the availability of well-described plant genetic resources. However, differences in the methods used to evaluate and describe germplasm held in genebanks in different countries, and in the accessibility of data, are a major obstacle, leading to the underutilization of these resources and negatively impacting future prospects (Egan *et al*, 2022). A better understanding of the stability and potential of measured traits can be achieved by considering data from different experiments, thereby improving the prospects of using genetic resources in novel breeding programmes (Cristobal *et al*, 2014). Consequently, genetic resource collections can become more utilized through enhanced cooperation and sharing not only seeds but also the accumulated knowledge gained over many years of resource regeneration and/or research (Guerra-García *et al*, 2021).

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Lentil (*Lens culinaris* Medik.) is one of the most important pulse crops and is an essential component of a balanced diet. Historically, the primary producers of lentils have been Asian countries and Canada. In Europe, lentils account for only 4% of the total area devoted to pulses. EU countries produce significantly fewer lentils than required to meet domestic consumption needs (TerresUnivia, 2021). France and Spain are the main producers and consumers of lentils within the European Union. The area harvested for lentils has doubled between 2010 and 2018 (Kaale *et al*, 2023). The expansion of lentil production in Europe depends on the development of novel, advanced, high-yielding and nutritionally improved varieties (Rajpal *et al*, 2023). The initial step in this process is a comprehensive review and analysis of the available genetic resources in genebanks to identify suitable sources for selecting and crossing in order to breed elite lines. The creation of special medium-core and mini-core collections allowed for more detailed study of traits, systematization of accumulated material and information, and generally better management of research (Bisht *et al*, 1998; Díez *et al*, 2018). However, starting this process from scratch is long and costly. It is, therefore, more expedient to utilize the material and information available in genebanks to accelerate the preliminary phase. Some genebanks, such as the Indian National Gene Bank (ICAR), which currently hosts 2,324 lentil accessions, have initiated this process. This genebank has characterized the entire collection for dozens of agromorphological traits to identify the most suitable accessions for use in breeding programmes (Tripathi *et al*, 2022).

The lentil collection of the National Plant Genetic Resources Centre of Ukraine (NCPGRU) in Kharkiv was established in 1993 and currently comprises 1,140 accessions, which were collected through interbank exchanges, independent expeditions and collaboration with breeders (Kobyzeva *et al*, 2011; Kir'yan *et al*, 2014; Vus *et al*, 2020c). All genotypes were subjected to a comprehensive study over three years, during which their main phenotypic, morphological and agronomic characteristics were outlined. Based on the collected data, a basic collection, an educational collection and a reference basic set were created and registered (Bezuhla and Kobyzeva, 2021). Accessions that successfully completed the research cycle and exhibited specific traits were included into a specialized trait-oriented database. This database served as the source of information for the first exchange of lentil seed material between French researchers and NCPGRU, which took place in 2019. This exchange facilitated evaluating the performance of selected samples under different climatic conditions. This also enabled the assessment of the potential of using additional data from the donor genebank for a more comprehensive analysis of the genotypes. Developing methods for comparing accessions despite the differences in cultivation and research techniques, as well as the soil and climatic characteristics of the regions, was particularly significant.

Information on accessions from the NCPGRU database collected during the period from 1993 to 2016 was processed for a preliminary evaluation and recommendation of genotype characteristics and a preliminary forecast of their use in breeding programmes. The results of field studies conducted in Ukraine (2019) and France (2021) were used to assess the accuracy of the provided forecasts, clarify the outcomes of the research, and refine the methodological approaches employed in such studies.

The aim of this research was to analyze data from the NCPGRU traits database to identify the most promising sources for breeding. The results were evaluated through trials conducted in two ecogeographical locations. The methods used took into account the specificity of each trial in terms of experimental design and choice of standards.

Materials and methods

Plant material

A total of 37 lentil (*Lens culinaris* Medik.) accessions from NCPGRU were included in this study, 29 of them belong to the subsp. *microsperma* (100-seed weight $<$ 4.5g), while the rest belongs to subsp. *macrosperma* (100-seed weight $>$ 4.5g). The accessions under study were from 19 countries (Table 1).

Transferring of lentil accessions was accompanied by SMTA agreement, according to the terms of the International Treaty on Plant Genetic Resources for Food and Agriculture (FAO, 2009).

For the list of 37 lentil accessions, which were transferred to the genebank of France (INRAE, Dijon), a preliminary evaluation was carried out according to the characteristic database of the genebank of Ukraine. Preliminary recommendations for their use were provided, together with a comparative assessment of their resistance to stress factors and potential productivity in comparison with standard accessions, based on phenotypic data during historic regenerations (1993-2016). In order to verify the applicability of the given prediction based on preliminary data from the genebank of Ukraine, two studies were conducted in Ukraine (2019) and in France (2021).

Figure 1 illustrates the complete data set, showing the accessions and the years in which they were evaluated.

Field trials

The field trials in Ukraine were carried out at the Plant Production Institute of named after V. Ya. Yuriev, Kharkiv, Ukraine (PPI NAAS) at the Laboratory of Genetic Resources of Grain Legumes - Elitne village, Kharkiv district, Kharkiv region (49°59'31"N, 36°26'55"E; 95m above sea level). A 4-year crop rotation was applied with winter wheat being systematically grown as the preceding crop for lentil. The experiments were conducted according to the *Methodical rec-*

Table 1. List of lentil accessions used in this study. The accession ID has been provided here to facilitate reference to the accessions. Genebank ID, accession name, country of origin and subspecies information are provided according to the National Plant Genetic Resources Centre of Ukraine (NCPGRU) database. The accessions were grouped in four clusters, for more details on clustering see Figure 4.

Accession ID	Genebank ID	Accession name	Country of origin	Subspecies	Cluster
1	UD0600006	CF 17-5	Morocco	*microsperma*	3
2	UD0600007	MEL M 5	France	*microsperma*	1
3	UD0600028	-	Ethiopia	*microsperma*	3
4	UD0600036	Krasnohrads'ka 49	Ukraine	*macrosperma*	2
5	UD0600052	Stepova 244	Ukraine	*microsperma*	4
6	UD0600065	Giza 9	Egypt	*microsperma*	1
7	UD0600084	Anicia	France	*microsperma*	4
8	UD0600086	MEL C 4	Morocco	*microsperma*	4
9	UD0600090	Dieu printemp foncé	France	*microsperma*	1
10	UD0600091	Du Puy	France	*microsperma*	4
11	UD0600092	Spatz Albinzée	France	*microsperma*	1
12	UD0600095	Ge IC-P	Hungary	*microsperma*	1
13	UD0600103	-	France	*macrosperma*	1
14	UD0600112	Krasnohrads'ka 250	Ukraine	*macrosperma*	2
15	UD0600119	-	France	*macrosperma*	1
16	UD0600131	Gornostepnaia	Armenia	*macrosperma*	1
17	UD0600145	Code 35	Morocco	*microsperma*	4
18	UD0600163	-	France	*macrosperma*	2
19	UD0600203	Flip 86-38L	Syria	*microsperma*	3
20	UD0600248	-	Syria	*macrosperma*	1
21	UD0600347	ILL 481	Lebanon	*microsperma*	3
22	UD0600403	Nadejda	Bulgaria	*microsperma*	4
23	UD0600423	CDC Sunrise	Canada	*microsperma*	1
24	UD0600437	-	Israel	*microsperma*	4
25	UD0600443	-	Iran	*microsperma*	4
26	UD0600444	-	Ethiopia	*microsperma*	1
27	UD0600468	-	France	*macrosperma*	2
28	UD0600490	-	Afghanistan	*microsperma*	1
29	UD0600496	-	Mexico	*microsperma*	2
30	UD0600521	-	China	*microsperma*	1
31	UD0600530	Slovyanka	Russia	*microsperma*	2
32	UD0600550	-	Bulgaria	*microsperma*	4
33	UD0600563	-	Ethiopia	*microsperma*	4
34	UD0600614	Precoz	Brazil	*microsperma*	1
35	UD0600638	Eston	Canada	*microsperma*	4
36	UD0600686	-	France	*microsperma*	2
37	UD0601021	Beluga	Israel	*microsperma*	1

ommendations for studying the genetic resources of grain legumes (Kobyzeva *et al*, 2016). The area of each experimental plot was 1m^2, and the sowing design was 20cm × 10cm, 6 rows of 10 plants each, or 60 plants per plot. The seeds were hand sown. Weeds were removed manually. In accordance with the methodology of genetic resources studies, and considering that no replicates were included, a block of standards sown every 20 plots was systematically added for the correct evaluation of the accessions in the field. For lentil evaluation in NCPGRU, the three historically used standard accessions were included in each standard block, namely Stepova 244 (UD0600052), Krasnohrads'ka 49 (UD0600036) and Krasnohrads'ka 250 (UD0600112).

Field trials in France were conducted at the Epoisses Experimental Unit (U2E), managed by INRAE, in Bretenière, France (05°05'57"N, 47°14'11"E; 210m above sea level). Agroecological management practices were applied. Weeds were removed manually. Seeds were sown mechanically and he experimental plot consisted of three rows of 1m each with 20 seeds per row, or 60 plants per plot. Three replications per accession with Anicia (UD0600084) and Beluga (UD0601021) were used as standard accessions.

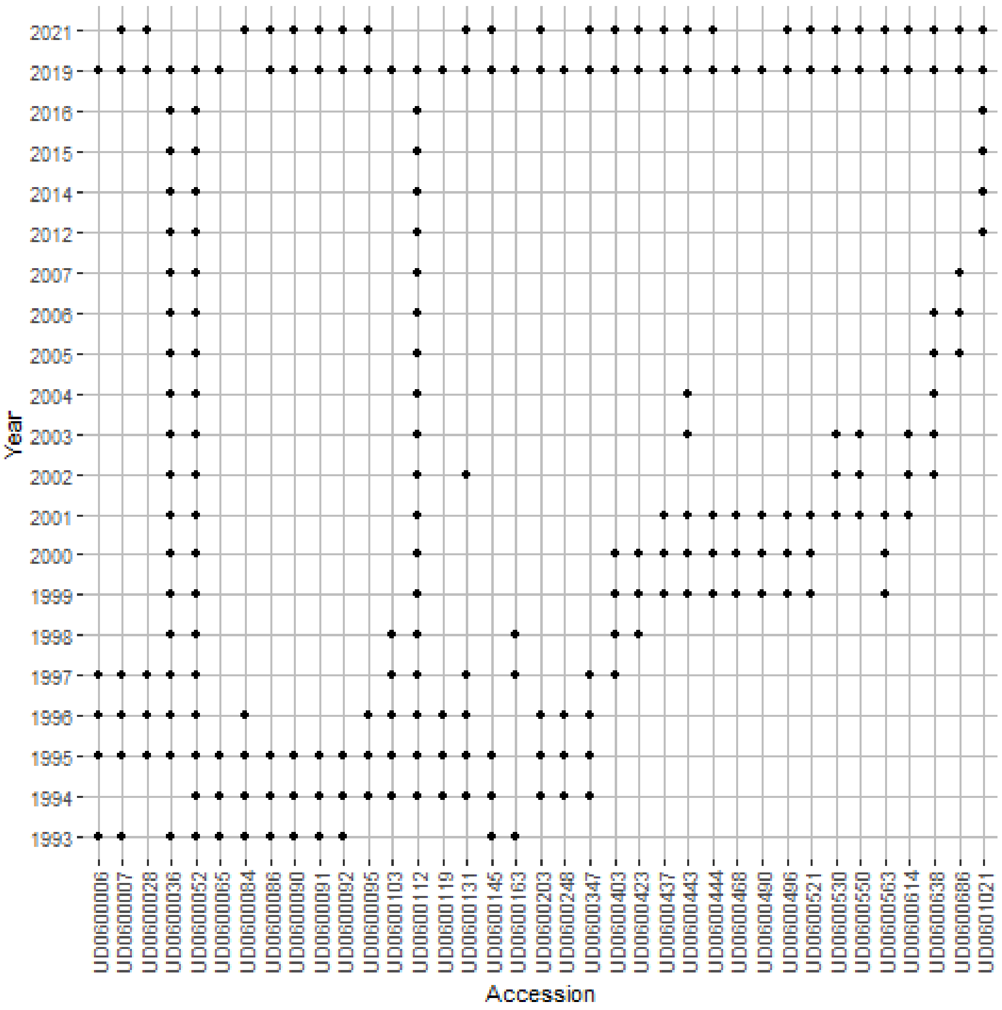

Figure 1. UpSet plot showing the years in which each lentil accession considered in this study was sown in the field for seed multiplication and phenotyping, including historical data (1993–2016 in Ukraine) and field trials conducted in 2019 in Ukraine and 2021 in France. For example, phenotyping data for UD0600006 are available for 1993, 1995, 1996, 1997 and 2019.

Plant phenotyping

Traits were characterized according to the lentil crop ontology (Agrawal, 2016). Phenological and agronomic traits were assessed on each accession. Phenological traits included days to 50% of flowering and pod filling period. Agronomic or yield component traits included seed yield per area (kg/m^2), seed yield per plant (g), number of seeds per plant, height of the lowest pod (cm), plant height (cm) and weight of 100 seeds (g). To allow genotype comparison in a representative way, each individual yield was normalized compared to the median of the standard accessions (CY, %) according to the formula below:

$CY = Ya/Ym*100$, (1)

where CY is the relative productivity (%), Ya is the accession's yield (kg/m^2), and Ym is the median of standards' yield (kg/m^2).

Statistical analysis

Statistical data processing and visualisation were carried out using R software version 4.2.2 (R Core Team, 2023) and in particular the following packages: Tidyverse and Rlang (Henry and Wickham, 2023), Openxlsx (Schauberger and Walker, 2022), Ggplot2 (Wickham, 2016), Cluster (Maechler *et al*, 2022), Metan (Olivoto and Lúcio, 2020) and Factoshiny (Vaissie *et al*, 2023).

Results

NCPGRU manages the *ex situ* conservation of 1,140 lentil accessions. Each of these accessions has undergone three years of mandatory field and laboratory research since 1993. Some have been grown in additional years to produce new seed lots and collect phenotyping data. All the information collected is stored in a database designed for this specific use. This study illustrates the use of historic genebank information on a subcollection of 37 lentil accessions from 19 countries. These accessions were requested by and made available to a research team in France in 2019. Following seed distribution, these accessions were evaluated in the field in Ukraine in 2019 and in France in 2021. The main objective was to show how access to the phenotyping data collected between 1993 and 2016 can provide valuable information on the potential of the accessions to be used for research and breeding, including outside Ukraine.

Comparison of weather conditions in trials in Ukraine (1993–2019) and France (2021)

The sowing dates of lentil accessions in Kharkiv, Ukraine varied each year depending on weather conditions. In general, sowing took place between the end of March and the beginning of May. Plants matured between the end of July and the beginning of August and the average length of the vegetation period was 84 days Average daily temperatures during the lentil growing season in Ukraine in the years of our observations (1993–2016) ranged from 15.6 to 21.3°C. The average sum of temperatures during the lentil period of vegetation was 1,661.3°C, and the average sum of precipitation during the same period was 176.8mm (Figure 2).

Weather conditions in 2019, when the research trial was conducted in Ukraine, were characterized by high precipitation at the beginning of the growing season and intermittent showers during flowering and maturity. The end of the growing season featured a prolonged period of drought and high temperatures, which led to rapid desiccation of the plants and had a negative impact on seed filling.

In Bretenière, France (Bourgogne-Franche-Comté region), in 2021, sowing took place on 25 February and germination was observed on 10 March. The plants reached maturity at the beginning of July, with an average vegetation period of almost 119 days. The weather conditions in the region during this year were atypical, exhibiting a greater degree of dryness and heat than is typical for a multi-year period. The weather conditions that accompanied the study in France were characterized by a lower temperature (average sum 1,528.1°) and a significantly higher amount of rainfall (258.1mm) during the growing season, compared to most of the trials conducted in Ukraine (Figure 2).

Analysis of the lentil yield data collected in Kharkiv, Ukraine in the period ranging from 1993 to 2016 and 2019 showed a rather high variability due to a wide range of weather conditions over the study years (Figure 3). The years with high productivity were 2006 and 2015, while the years with the lowest seed production were 1996 and 2014. This allowed a comprehensive assessment of the stability of the performance of the lentil accessions.

Analysis of Ukrainian genebank data

The database of the NCPGRU lentil collection includes results from multiple years of research in the field and in the laboratory. The analysis of variance for 37 lentil accessions from the genebank database in this study, which provided insights into the genetic diversity and variability, is presented in Table 2.

Considering ten agronomic traits with a significant coefficient of variation (ranging from 11.7 to 75.92%), it was found that the duration of the growing season (11.7%) and the number of days to flowering (15.87%) were the most stable traits. Conversely, seed yield per plot exhibited the highest coefficient of variation (75.92%).

The generalization of the long-term data (1993–2016) of the genebank of Ukraine was carried out using principal component analysis (PCA) (Figure 4).

PCA of agronomic traits (seed yield per plant (P1), weight of 100 seeds (P2), seed yield per area (P3), number of seeds per plant (P6), plant height (M1), height of the lowest pod (M2), and relative yield to standards (CY)) and weather parameters including sum of effective temperatures (and sum of precipitations by different phenophases) revealed that the first two components accounted for over 50% of the total variance. A strong correlation was observed between yield components and weather conditions (both total and relative yield, weight and number of seeds, height of attachment of the lower pod). For the second axis, the most significant indicator was the duration of the growing season and the sum of temperatures experienced during this period.

The qualitative factor map (Figure 4B) illustrates that Dimension 1 opposes individuals located on the right of the graph, characterized by strongly positive coordinates on the axis to individuals characterized by strongly negative coordinates on the axis (left of the graph). This group, characterized by positive coordinates on the axis, showed high values for variables like seed yield per plant (P1), seed yield per area (P3), relative yield to standards (CY), number of seeds per plant (P6), plant height (M1), height of the lowest pod (M2), days from germination to full maturity (F5), sum of effective temperatures during the vegetation period (S_T_C), days to 50% of flowering (F8) and days to 50% of flowering (F2). The variables are ranked from the strongest to weakest.

A K-means cluster analysis was performed to identify groups of lentil accessions based on the similarity of their agronomic characteristics. The 37 accessions were grouped into four clusters with no direct relationship between cluster separation and the geographical origins of the accessions (Table 1, Figure 4). Cluster 1 and Cluster 4 were the largest with 15 and 10 total accessions, respectively. The average indicators for each cluster are summarized in Table 3.

Cluster 4 is the most promising for breeding. The accessions have a high mean yield (0.156kg/m^2) and a high mean percentage yield compared to the standards (103.45%) and all belong to the subspecies *microsperma*. Three of them are from France. The complete characteristics of the lentil accessions from this cluster, according to the most important agronomic traits, are given in Table 4.

In addition, Cluster 2, which combines seven accessions of the *macrosperma* type, is important for further breeding processes. Cluster 2 includes two standard accessions from Ukraine and five landraces – three from France, one from Mexico and one from

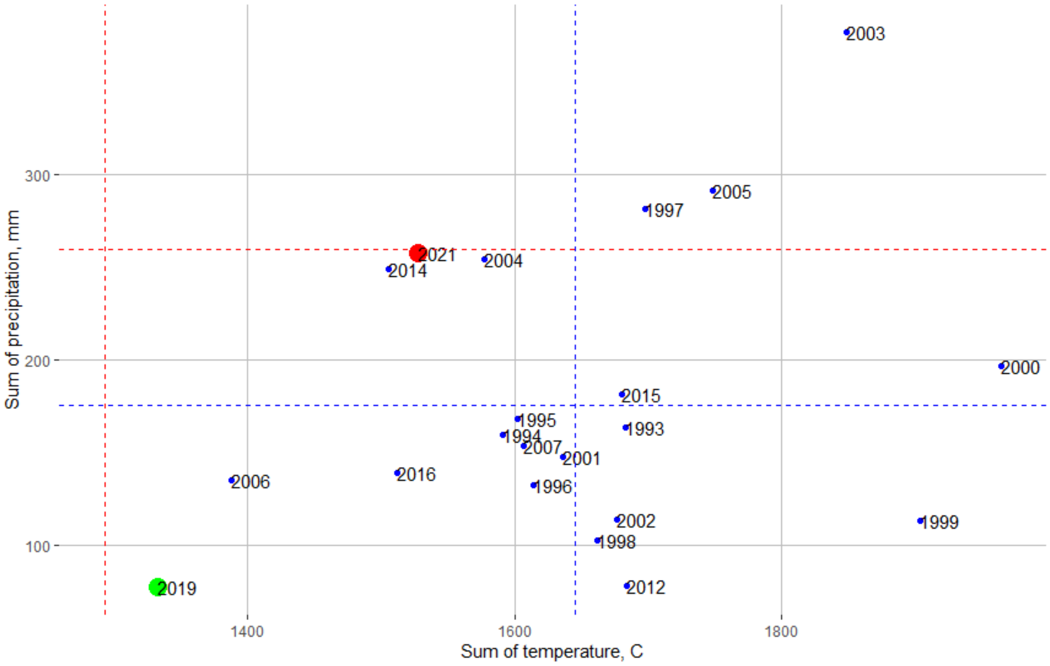

Figure 2. Sum of temperature (°C) and sum of precipitation (mm) during the lentil vegetative period in Ukraine (1993–2016 and 2019) and France (2021). The red dot refers to the year when the accessions provided by the National Plant Genetic Resources Centre of Ukraine (NCPGRU) were evaluated in the field in France. The green dot refers to the year when the maximum number of accessions evaluated in France were considered for seed multiplication and phenotyping in Ukraine. The dashed lines correspond to the average sum of temperature and sum of precipitation: blue for Kharkiv, Ukraine (1993–2016) and red for Breteničre, France (2011–2021).

Table 2. Analysis of variance for 37 lentil accessions from the National Plant Genetic Resources Centre of Ukraine (NCPGRU) database (1993–2016). SD, standard deviation; CV, coefficient of variation; ***, statistically significant at $P < 0.001$.

Traits	Min–max	Mean	SD	CV, %	Source of variation	
					Genotype	Year
Plant height (M1), cm	0–80	37.54	13.76	36.66	4.617***	11.491***
Height of the lowest pod (M2), cm	0–26	14.43	6.11	42.35	3.496***	8.842***
Number of seeds per plant (P6)	0–329	93.3	69.08	74.04	5.116***	6.319***
Weight of 100 seeds (P2), g	0–87.6	39.45	19.92	50.50	12.412***	6.156***
Seed yield per plant (P1), g	0–9.5	2.96	1.96	66.24	3.496***	8.842***
Seed yield per area (P3), kg/m^2	0–0.43	0.137	0.104	0.076	4.978***	16.621***
Relative productivity (CY), %	0–457.45	145.44	110.42	75.92	4.978***	16.621***
Days from germination to 50% flowering (F2)	25–56	42.6	6.76	15.87	14.04***	29.13***
Days from 50% flowering to full maturity (F8)	13–66	41.38	8.49	20.51	2.479***	18.948***
Days from germination to full maturity (F5)	61–108	83.98	9.83	11.70	7.231***	26.837***

Table 3. Agronomic traits characterizing the four clusters of lentils (1993–2016) (means for cluster).

Cluster	Plant height, cm	Height of the lowest pod, cm	Number of seeds per plant	Weight of 100 seeds, g	Yield of seeds per plant, g	Yield per plot, kg/m^2	Relative yield to standards, %
1	33.39	14.91	70.63	3.4	1.85	0.080	59.63
2	42.91	19.56	59.86	5.9	2.71	0.110	85.9
3	25.44	8.95	23.13	3.2	0.88	0.024	23.69
4	39.66	15.17	115.41	2.9	3.15	0.156	103.45

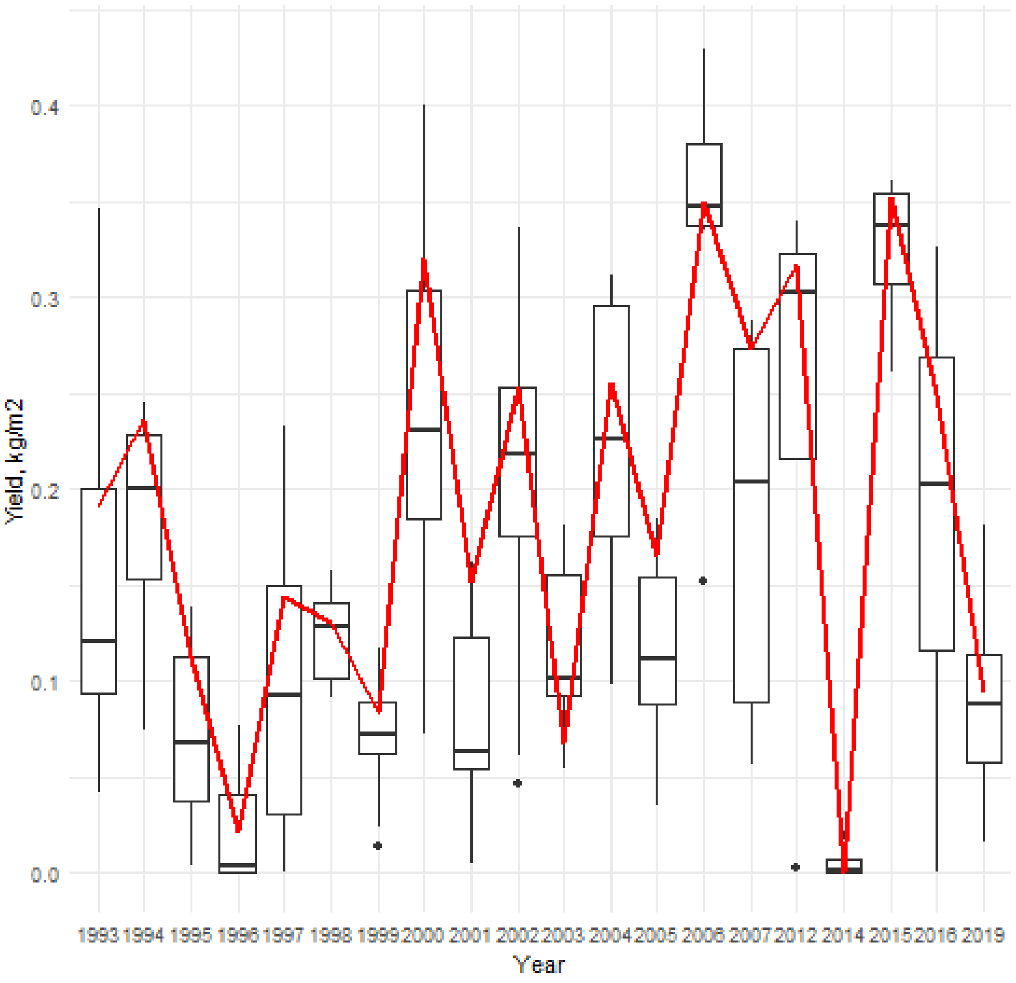

Figure 3. Boxplots showing seed yield (kg/m^2) of lentil accessions per research year in Kharkiv, Ukraine. The red line represents the median of the yield of three standard accessions, Stepova 244 (UD0600052), Krasnohrads'ka 49 (UD0600036) and Krasnohrads'ka 250 (UD0600112), regularly sown and phenotyped in each year.

Table 4. Agronomical traits of ten *microsperma* lentil accessions composing Cluster 4 (National Centre for Plant Genetic Resources of Ukraine (NCPGRU), 1993–2016).

Accession ID	Plant height, cm	Height of the lowest pod, cm	Number of seeds per plant	Weight of 100 seeds, g	Yield of seeds per plant, g	Yield per plot, kg/m ^{2}	Relative yield to standards, %
UD0600052	44.5	15	187	2.6	3.9	0.196	97.94
UD0600084	32.2	13.3	182.7	2.7	3.75	0.161	144.72
UD0600086	34.5	14.5	113.5	2.6	2.95	0.168	104.1
UD0600091	45.7	13.7	55.9	2.5	2.9	0.149	97.13
UD0600145	36.3	20	88	3.9	3.6	0.163	85.65
UD0600403	34.8	12.4	75	3.4	2.4	0.156	107.76
UD0600437	40.6	18.4	109	2.7	2.6	0.090	78.9
UD0600443	34.3	10.25	122	3	3	0.152	92.25
UD0600550	52.1	18.6	98	2.5	2.55	0.108	109.54
UD0600638	41.6	15.5	123.00	3.1	3.8	0.217	116.5

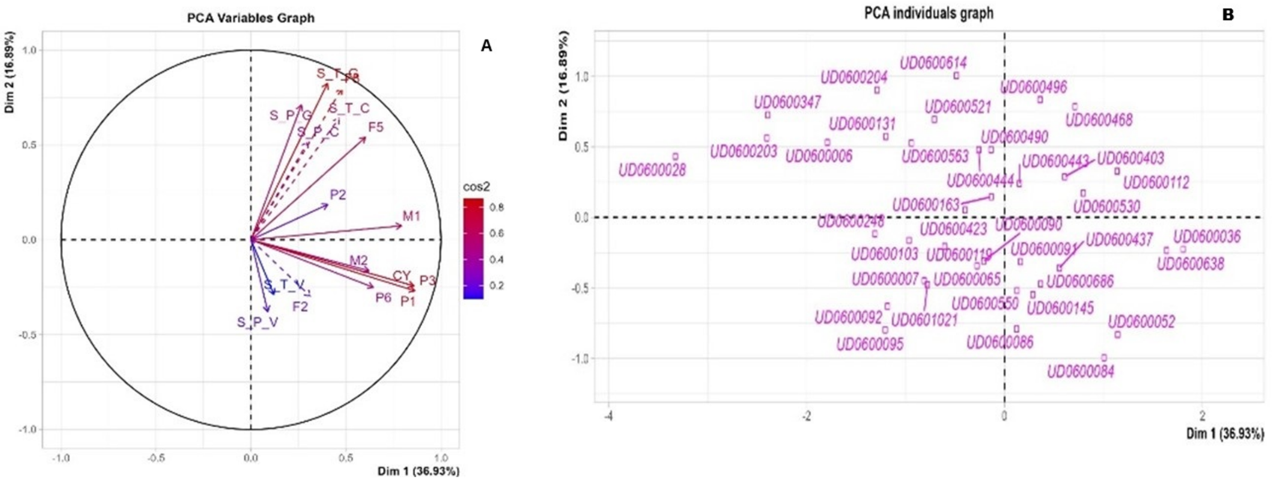

Figure 4. Principal component analysis of phenological, agronomic and meteorological parameters. A, Variables factor map; B, Qualitative factor map. Phenology traits include: F2, days to 50% of flowering; F5, days from germination to full maturity; F8, days from 50% of flowering to full maturity. Agronomic traits include: P1, seed yield per plant, g; P2, weight of 100 seeds, g; P3, seed yield per area, kg/m^2; P6, number of seeds per plant; M1, plant height, cm; M2, height of the lowest pod, cm; CY, relative yield to standards. Weather parameters: S_T_C, sum of effective temperatures during the vegetation period; S_P_C, sum of precipitation during the vegetation period; S_T_G, sum of temperature during the pod filling period; S_P_G, sum of precipitation during the pod filling period; S_T_V, sum of temperature during the period from germination to the beginning of flowering, S_P_V, sum of precipitation during the period from germination to the beginning of flowering.

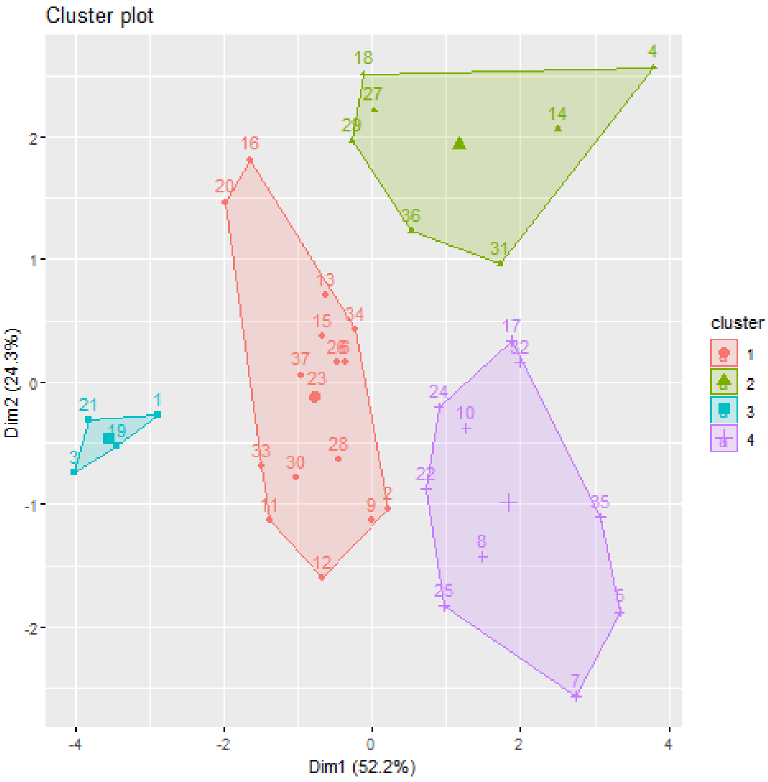

Figure 5. Cluster plot highlighting four groups of lentil accessions based on seven agronomical traits (1993–2016).

Russia. Table 5 shows the complete characteristics of the lentil accessions from this cluster.

Comparative analysis of Ukrainian genebank data, and data from field experiments in Ukraine (2019) and France (2021)

A comparison of the duration of the phenophases in France and Ukraine, conducted in conjunction with the long-term observations of the genebank, showed that despite the significantly earlier sowing date in France, the growing season was not significantly longer, with an average of 118.72 days in France versus 105.24 days in Ukraine (2019). The experimental year in Ukraine (2019) was characterized by particularly unfavourable conditions for lentil cultivation, with a much shorter growing season than usual (63.94 days).

The results of the field experiments in Ukraine (2019) and France (2021) are presented in Table 6. We found that the examined set of accessions (37 genotypes) showed a significant decrease in productivity, in both Ukraine (2019) and France (2021) compared to the characteristic indicators of the genebank for the evaluation period 1993–2016 (Table 2), most likely due to weather conditions.

The average seed yield per plot in France was almost half that in Ukraine (0.042kg/m^2 and 0.089kg/m^{2}respectively), but this reflected the typical conditions of the year of the study and the design of the experiment, as can be seen from the fact that the relative yield to the standard is almost the same in both experiments (95.45% and 94.57%). The most stable trait in both experiments (Ukraine and France), as in the analysis of long-term data of NCPGRU, was the duration of the growing season. The most variable features were those representing yield components (Table 6).

The biplot analysis of the genotype×environment interaction and the ranking of genotypes revealed a notable discrepancy in environmental conditions between the long-term observations conducted by NCPGRU (1993–2016) and the experimental years in Ukraine (2019) and France (2021). This enabled a comprehensive evaluation of the selected accessions, with UD0600638 Eston (Canada) and UD0600052 Stepova 244 (Ukraine) identified as the most closely aligned with the ‘ideal’ genotype (Figure 5).

The most informative environment, as expected, turned out to be the conditional environment of genebank (information from the database of NCPGRU). The ranking of genotypes in relation to the ’ideal’ genotype should be based on two criteria: average efficiency and stability across environments. The ideal genotype, represented by the point in the centre of the concentric circles, represents ‘absolute stability’ in the positive direction and has a vector length equal to the longest vectors of genotypes on the positive side of the highest average seed yield per plot. Consequently, genotypes that are closer to the ideal genotype are more desirable. By comparison with the ideal genotype, six accessions were selected, located in the first three rounds of the concentric circles in the graph (Figure 5): UD0600638, UD0600052, UD0600145, UD0600403, UD0600084, UD0600086.

Significant differences in environmental conditions were analyzed by biplot analysis based on the indicator of relative yield to the standard (standard accessions were removed from the ranking). This approach gave a much larger range of variation in all three environments (Figure 6).

As a result, eight accessions were selected within the circles around the ideal genotype. Five of these accessions belong to Cluster 4 and one to Cluster 2 (Table 7).

Discussion

The evaluation of genetic resources and the identification of the most promising parental lines is crucial in the development of new varieties (Sivaraj *et al*, 2022). The exchange of seed material and information on accessions and their characteristics is not always optimal due to the locations of genebanks in a wide range of climatic zones. It is known that environmental conditions have a significant influence on the yield of lentil genotypes (Khatun *et al*, 2022). The study of the lentil gene pool has a long history in Ukraine (Bezuhla and Kobyzeva, 2021), while it is a relatively new endeavour in France. It was therefore decided, upon seed material request, to accompany seeds with related phenotyping data to increase their utility use efficiency. The initial phase of the study involved gathering historic data on these accessions from NCPGRU, collected during previous regeneration cycles. It became evident that significant discrepancies in weather patterns and regional variations in agricultural technology and applied methods preclude a straightforward comparison of lentil productivity. Consequently, a comparison of the relative yield against the median of the standards included in field trials was conducted, accompanied by an assessment based on a set of characteristics.

The principal components analysis of the NCPGRU data revealed that PC1 was influenced by yield indicators, while PC2 was affected by weather conditions. The combination of temperature and precipitation exerted the most significant influence on the generative phase of lentil development, thereby determining the duration of the growing season in general (Maphosa *et al*, 2023). The characteristics of yield per plot (direct and relative to standards), the height of pods attachment, and the weight and number of seeds per plant exhibited the strongest correlation. This finding aligned with literature data (Hussain *et al*, 2022; Ilyas *et al*, 2024). Accessions situated in the lower right quadrant demonstrated a greater contribution in terms of yield, while those in the upper right quadrant exhibited a greater contribution in terms of the length of the growing season.

The results of the ANOVA indicated that the duration of phenophases, yield, plant height and the attachment of the lower pod bean exhibited a greater degree of

Table 5. Agronomic traits of seven *macrosperma* lentil accessions composing Cluster 2 (National Centre for Plant Genetic Resources of Ukraine (NCPGRU), 1993–2016).

Accession ID	Plant height, cm	Height of the lowest pod, cm	Number of seeds per plant	Weight of 100 seeds, g	Yield of seeds per plant, g	Yield per plot, kg/m^2	Relative yield to standards, %
UD0600036	48.4	22.9	62.5	6.7	4.9	0.188	104.93
UD0600112	44	18.7	60.5	6.9	4.05	0.160	100
UD0600163	40	18	36	6.9	2.05	0.077	67.44
UD0600468	38.8	19.1	47.5	6.4	1.85	0.073	81.27
UD0600496	40	19.9	39.5	5.2	1.45	0.734	72.7
UD0600530	46.3	19.7	98.5	4.5	2.25	0.106	111.34
UD0600686	42.9	18.6	74.5	4.6	2.4	0.089	63.64

Table 6. Comparison of the main traits of lentils in experimental years in Ukraine (2019) and France (2021). SD, standard deviation; CV, coefficient of variation; ***, statistically significant at $P < 0.001$.

	Ukraine				France			
Traits	**Min–max**	**Mean**	**SD**	**CV, %**	**Min–max**	**Mean**	**SD**	**CV, %**
Plant height, cm	25–39	31.75	3.68	11.59	10–35	21.3	6.12	28.73
Height of the lowest pod, cm	8–23	15.86	3.26	20.55	5–23	12.58	3.97	31.56
Number of seeds per plant	9–117	48.61	30.69	63.14	1.3–128.77	49.9	27.85	55.81
Weight of 100 seeds, g	2.13–6.6	3.77	1.37	36.45	0–2.65	1.64	0.34	20.73
Yield of seeds per plant, g	0.3–3	1.54	0.76	49.35	0–2.6	0.83	0.49	59.04
Yield per plot, kg/m^2	0.016–0.181	0.089	0.05	52.99	0–0.117	0.042	26.22	61.90
Relative yield, %	16.7–192.55	94.57	50.18	53.06	0–266.7	95.45	59.81	62.66
Days from germination to 50% flowering	13–44	39.47	5.22	13.23	68–90	83.88	3.45	4.11
Days from 50% flowering to full maturity	19–64	24.47	7.25	29.63	25–44	34.84	3.60	10.33
Days from germination to full maturity	63–77	63.94	2.32	3.63	110–128	118.72	4.25	3.58

Table 7. Characteristics of the most promising lentil accessions revealed across trials conducted in Ukraine and France.*, Accessions were not evaluated in France.

Accession ID	Seed yield per plot, kg/m^2			Relative yield per plot from median of standards (CY), %			Cluster
	Genebank (median of years)	**Ukraine (2019)**	**France (2021)**	**Genebank (median of years)**	**Ukraine (2019)**	**France (2021)**	
UD0600006*	0.021	0.140	-	13.03	148.94	-	3
UD0600065*	0.082	0.136	-	42.71	144.68	-	1
UD0600086	0.197	0.099	0.034	102.87	105.32	76.72	4
UD0600145	0.168	0.158	0.044	71.04	168.09	99.29	4
UD0600437	0.092	0.088	0.047	64.17	93.62	106.75	4
UD0600530	0.102	0.110	0.047	105.66	117.02	106.16	2
UD0600550	0.096	0.167	0.031	177.66	78.63	69.92	4
UD0600638	0.252	0.181	0.030	111.43	192.50	68.38	4

variation in response to the prevailing conditions of the year (Lázaro *et al*, 2001). In contrast, seed size and time to 50% flowering were largely influenced by genotype (Bhartiya *et al*, 2015).

Cluster analysis allows the grouping of a large number of genotypes based on a list of characteristics and the selection of a group of accessions that meet the requirements of a specific breeding programme (Ahamed *et al*, 2014; Vus *et al*, 2020a; Zayed *et al*, 2022; Amit *et al*, 2023). The clustering of the 37 accessions from this study allowed the identification of a cluster of lines (Cluster 4; Table 3) as the most promising for breeding with high and stable productivity. All accessions of this cluster were *microsperma* type and had a higher yield than accessions of *macrosperma* type, consistent with results from Mohammed *et al* (2019) and Preiti *et al* (2024) who suggested that this is caused by earliness of this type of lentils. Cluster 4 included well-known accessions such as UD0600638 Eston (Canada), the most popular Canadian variety. However, this one is also susceptible to Ascochyta blight (Sari *et al*, 2018). This cluster also contained accession UD0600052 Stepova 244 (Ukraine) – a reference for yield stability in Ukraine (Bezuhla and Kobyzeva, 2021), which has been

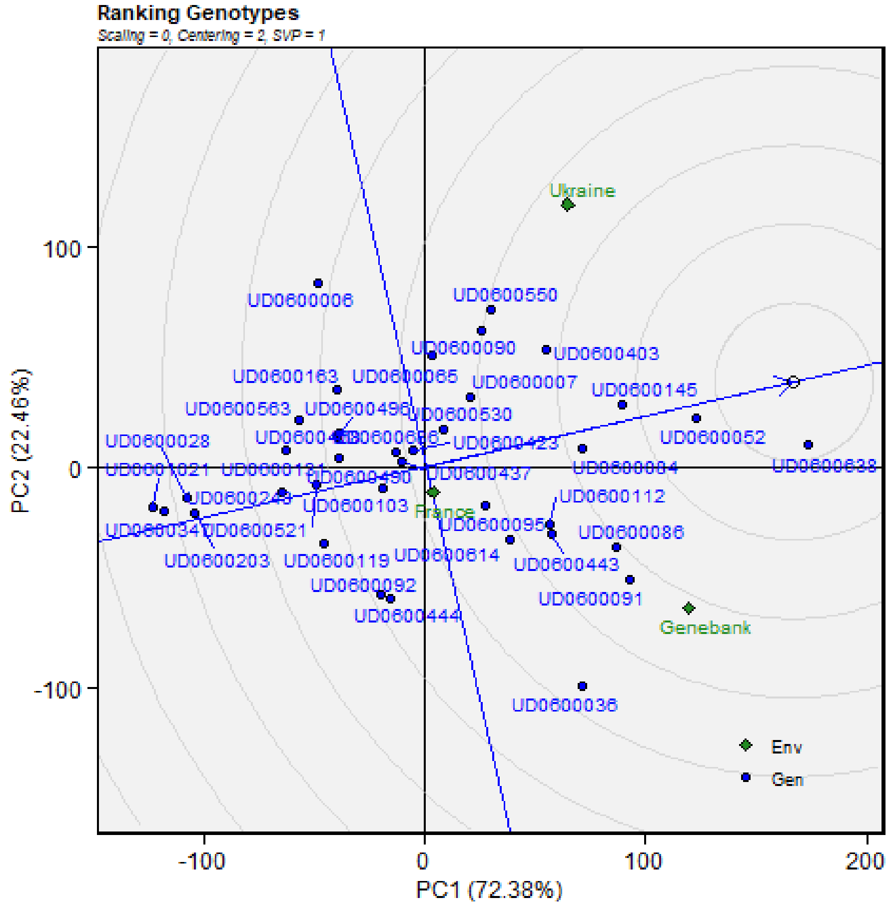

Figure 6. Biplot analysis of genotype-environment interaction by yield of seeds per plot. Ranking genotypes relative to the 'ideal' genotype (centre of the concentric circles). Green dots are environments and blue dots are genotypes.

used in many studies aiming at understanding drought tolerance (Vus *et al*, 2020b) or long-term seed storage behaviour (Zadorozhna *et al*, 2015). The Bulgarian variety Nadejda (UD0600403) is largely used in research by Bulgarian scientists. Its resistance to four of the seven pathotypes of Ascochyta blight known in the country has been established (Stanoeva and Koleva, 2017), but also the high sensitivity to changes in weather conditions was noted (Milev, 1999; Tonev *et al*, 1999). This was also confirmed in our research: in France, the variety yielded significantly lower than the standard (13.52%), while in Ukraine it performed well (171.28%).

Large-seeded lentil genotypes in Cluster 2 (Table 4) included two Ukrainian reference accessions (UD0600036 and UD0600112) and UD0600530 Slovyanka, which has been identified as a source of the highest first pod (Kobyzeva *et al*, 2012). This feature is an important character for mechanization in lentil, as it shows high variability depending on growth conditions and can cause significant yield losses (Gaad *et al*, 2018; Kuzbakova *et al*, 2022). Accession UD0600444 is part of a set of lentils with high seed nutritional value (Vus et al., 2020a). It should be noted that some genotypes in this cluster, which have important breeding traits such as high weight of 100 seeds (UD0600163) or high number of seeds per plant (UD0600686), had a high susceptibility to environmental changes and a low yield compared to the standard (27.42 and 32.6%, respectively), consistently with results from (Mohammed *et al*, 2019).

The ranking of lentil accessions under different environmental conditions – using the genebank's median data as one environment point, along with data from field trials conducted in Ukraine (2019) and France (2021) – was carried out using biplot analysis based on the feature 'seed yield per plot'. It was noted that the years of research trials in Ukraine and France were significantly different from the long-term observations of NCPGRU. In France, a low variability of lentil accessions was observed, compared to Ukraine. According to the relationship to the 'ideal' genotype – represented by the centre of circles in the PCA – an ideal genotype should have both high mean performance and high stability across environments. Therefore, genotypes closer to the ideal are considered more desirable (Gedif and Yigzaw, 2014). Six lentil accessions located within the first three circles from the centre were selected as more promising for the three environments (Yan and Tinker, 2006; Khan *et al*, 2021). Significantly, two of them were standards: UD0600052 (in Ukraine) and UD0600084 (in France), with a consistently high yield in the study regions. All of these accessions were also identified earlier as promising

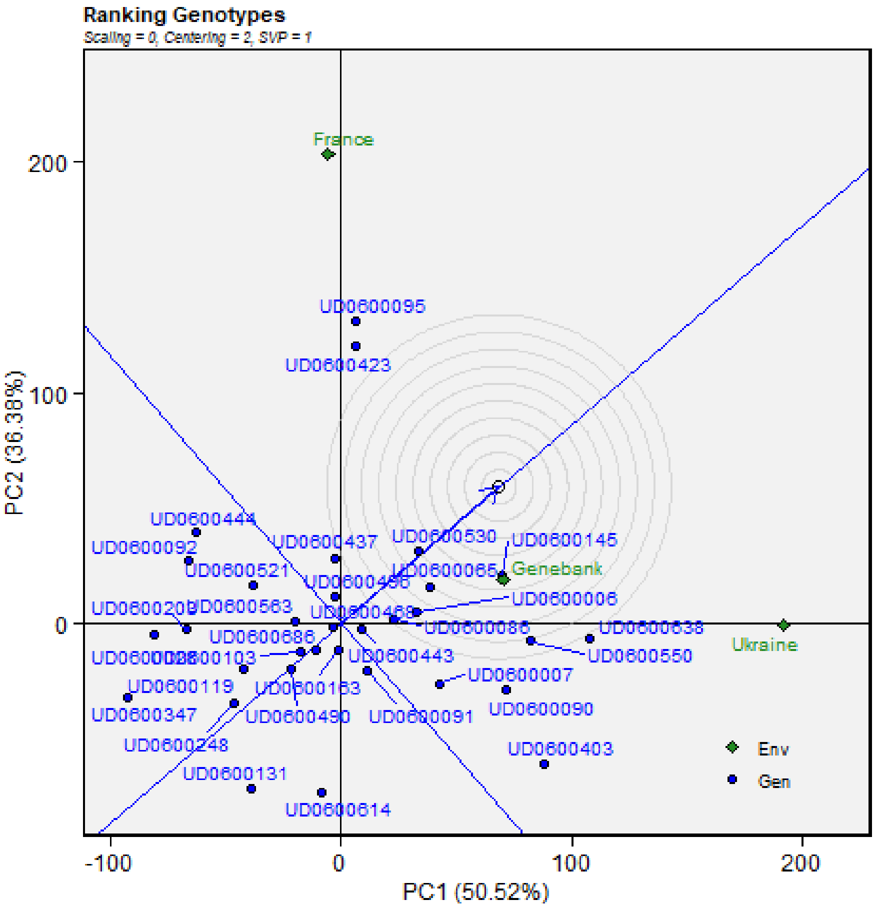

Figure 7. Biplot analysis of genotype-environment interaction by relative yield (without standards accessions). Ranking genotypes relative to the ‘ideal’ genotype (centre of the concentric circles). Green dots are environments and blue dots are genotypes.

by results of multi-year observations in NCPGRU (Cluster 4). ‘Stable’ genotypes are desirable only when they have high mean performances.

It should also be noted that accessions UD0600095 and UD0600423 showed the highest level of productivity in the conditions of the year of research in France. It has been confirmed (Bezuhla and Kobyzeva, 2021) that UD0600423 CDC Sunrise was determined as a source of two valuable features: high protein content and low cooking time.

Conclusions

The present study demonstrated how the valuable information stored in genebank databases is essential for supporting research and development, using an underutilised crop – lentil – as a key example. The performance of lentil accessions under different annual and agroclimatic conditions in Ukraine (Kharkiv region) allowed the identification of promising accessions that could be confirmed in parallel trials conducted in Ukraine and in France (Bourgogne-Franche-Comté region). Accessions that consistently outperformed the standards are recommended for use in breeding to create new lentil varieties.

Authors contributions

Nadiia Vus: Conceptualization, data curation, analyses conduction, investigation, methodology, resources, provision of study materials, validation, verification of the overall result replications, visualization, prepared the figures and tables, writing (original draft, review and editing); Olha Bezuhla: supervision, data curation, resources, provision of study materials, validation; Hervé Houtin: resources, validation, provision of study materials, verification of the overall result replications; Florence Naudé: resources, validation, provision of study materials, verification of the overall result replications; Antonina Vasylenko: analysis conduction the analyses, methodology, validation, verification of the overall result replications, writing (review and editing); Anthony Klein: resources, validation, verification of the overall result replications, provision of study materials; Oleh Leonov: data curation, analysis conduction, validation, verification of the overall result replications; Nadim Tayeh: supervision, conceptualization, data curation, provision of study materials, formal analysis, investigation, resources, validation, visualization, writing (review and editing), acquisition of financial support.

All authors read and approved the final manuscript.

Conflict of interest statement

The authors confirmed that no conflict of interest exists.

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