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Gamo and Gofa cattle, Ethiopia. Credit: B. Banjaw *et al*; Sorghum varieties, Sudan. Credit: I.M. El Tahir *et al;* Ñuña beans, Peru. Credit: Santa Cruz-Padilla *et al*.

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Editorial Office

ECPGR Secretariat
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Via di San Domenico 1
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Assessment of phenotypic diversity of Ñuña, a local common bean (*Phaseolus vulgaris* L.) from the northern Andes in Peru

Angel Esteban Santa Cruz-Padilla [*,a], **Jorge Luis Vásquez-Orrillo** [*,a], **Ricardo Manuel Bardales-Lozano** [b] **and Hipolito Murga-Orrillo** [c]

[a] *Instituto Nacional de Innovación Agraria (INIA), Estación Experimental Agraria Baños del Inca, Subdirección de Recursos Genéticos, Jirón. Wiracocha s/n, 06004, Los Baños del Inca, Perú*
[b] *Universidad Nacional de la Amazonía Peruana (UNAP), Departamento Académico de Suelos y Cultivos, Facultad de Agronomía, Jirón Nauta, 16002, Iquitos, Perú*
[c] *Universidad Nacional Autónoma del Alto Amazonas (UNAAA). Escuela Profesional de Ingeniería Agrónoma, Prol. Libertad 1220 - 1228 - Yurimaguas, Alto Amazonas, Loreto, Perú*

Abstract: Ñuña is a local type of common bean (*Phaseolus vulgaris* L.) cultivated in the Andean region of Peru. It stands out for its ability to expand and burst when roasted; however, its phenotypic diversity has not yet been fully explored. This study determined the phenotypic variability of Ñuña conserved in the Germplasm Bank of the National Institute of Agrarian Innovation of Peru. The analysis considered qualitative and quantitative traits, using multivariate statistics and comparison of means. Results revealed high phenotypic variability in both qualitative and quantitative traits. In qualitative traits, Multiple Correspondence Analysis found that the dark and light colours of the seed heads contributed most significantly to the variability of the accessions. Phylogenetic hierarchical analysis formed four clusters, representing 37% (I), 4% (II), 7% (III), and 52% (IV) of the accessions, respectively. For quantitative traits, Principal Component Analysis showed no discrimination between regions of origin but indicated a highly positive correlation between leaf length and width, and between pod length and width, as well as seed length, width, thickness and weight. Hierarchical analysis of quantitative characters also formed four clusters, representing 22% (A), 16% (B), 30% (C), and 31% (D) of the accessions, respectively. These clusters, analyzed for means comparison, showed significant differences ($p < 0.05$) with higher values in cluster B for pod length and width, and seed length, width, thickness and weight. Understanding the variability of the qualitative and quantitative traits of Ñuña is crucial for future genetic improvement studies aimed at achieving cultivars with desirable characteristics.

Keywords: Qualitative traits, quantitative traits, hierarchical analysis, legume, germplasm

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Introduction

Bean (*Phaseolus vulgaris* L.) is the most consumed grain legume worldwide. Its production has spread mainly to developing countries (De Ron *et al*, 2016; Nassary *et al*, 2020; Uebersax *et al*, 2023), for its contribution to dietary protein for more than 300 million people in rural and urban communities in East Africa and Latin America (Petry *et al*, 2015). The areas dedicated to bean production are 36 million hectares worldwide, of which 6 million are located in Latin America (FAOSTAT, 2022).

In Latin America, Ecuador and Peru are considered to be the places of origin of the bean (Kami *et al*, 1995). This gene pool was disseminated through independent

*Corresponding authors: Angel Esteban Santa Cruz-Padilla (asantacruz@inia.gob.pe), Jorge Luis Vásquez-Orrillo (jorge.vasquez.orrillo@gmail.com)

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domestication events, creating diverse landraces (Iwata-Otsubo *et al*, 2016). Within this genetic variability, one particular type is the Ñuña bean (Beem *et al*, 1992; Fernández *et al*, 2014), produced on a small scale by local producers in northern Peru.

The Ñuña plant is characterized by its indeterminate and climbing growth habit (Gamarra, 2021), reaching a height of more than 2 metres at the end of flowering (Gamarra *et al*, 2007). The pods are usually distributed along the entire length of the plant (Melo and Ligarreto, 2010) and contain between five and seven grains. These beans stand out for their protein content, which varies between 18% and 23% (Rodríguez *et al*, 2014). As with common beans, Ñuña presents high levels of other nutrients such as vitamins and minerals (Beem *et al*, 1992; Melo and Ligarreto, 2010).

The main characteristic of the Ñuña kernel when roasted for consumption is to explode and expand like popcorn, acquiring a soft consistency and a pleasant flavour similar to peanuts. This characteristic is associated with the presence of occluded intracellular and intercellular spaces that are forced to expand by the generation of water vapour during roasting (Beem *et al*, 1992).

The Ñuña has diversified under geographical restrictions, with production concentrated continuously in the Andean zone (National Research Council, 1989; Tohme *et al*, 1995; Otálora *et al*, 2006). It develops between the latitudes of 7°30' S and 19°30' S, and altitudes ranging from 2,000 to 3,000masl (Pearson *et al*, 2012). Temperatures in this region fluctuate between 10 and 30ºC (Llique, 1993), with a relative humidity of 60 and 75% (Hernández-López *et al*, 2013), annual rainfall of 500 to 3,000mm and a photoperiod of 8 to 14 hours (Llique, 1993).

In Peru, the Ñuña has a wide phenotypic variability that is distributed in the regions of Cusco, Ancash, Huánuco, Apurímac, Ayacucho, La Libertad and Cajamarca. The most significant genotypic variability is found in the latter region (Tohme *et al*, 1995), particularly in the province of Cajabamba, where there is a superior gene pool with varied productivity levels (Debouck, 1986; Franco and Hidalgo, 2003; SantaCruz-Padilla *et al*, 2021).

Despite the existence of morphological and molecular characterization studies of Ñuña (Cruz-Balarezo *et al*, 2009), there are few reports on the morphology of genotypes in northern Peru. Considering that ecogeographic factors influence the phenotypic and genetic characteristics of a species (Knight *et al*, 2005; Herben *et al*, 2012; Díez *et al*, 2013), it is hypothesized that the morphological characteristics of flowers, pods and grains of Ñuña contribute to discriminating the morphological variability of the accessions conserved in the Germplasm Bank of the National Institute for Agrarian Innovation (INIA) in Peru. Furthermore, it is considered that there is phenotypic variability related to the geographical area of origin. Therefore, this research aimed to determine the phenotypic variability of Ñuña conserved in the germplasm bank of INIA in Peru.

Material and methods

Origin of the Ñuña accessions

The Ñuña samples were collected from plots belonging to producers located in eight districts within the regions of La Libertad, Cajamarca, and Ancash in Peru, as detailed in Table 1 and illustrated in Figure 1. Most of the Ñuña germplasm collected in northern Peru was from the Cajamarca region (88 accessions), followed by the La Libertad region with 31 accessions and the Ancash region with 3 accessions (Table 1).

Location of the study

The study covered the period from December 2019 to July 2020 and was carried out at the Cochamarca Experimental Annex of the Baños del Inca Agricultural Experimental Station, Cajamarca (7.2756 S, 78.2186 W, 2,820masl, Figure 1). The study area exhibits a climatic classification corresponding to the tropical low montane dry forest (bs-MBT) category, determined according to the methodology of Holdridge (1947). Throughout the research period, mean, minimum and maximum temperatures were 14.3°C, 7.6°C and 21°C respectively, with a rainfall of 117.4mm (SENHAMI, 2020).

Soil chemical properties

A composite sample was extracted from the experimental area using an auger at a 0 to 30cm depth. This sample was treated at the Baños del Inca - INIA Soil, Water and Foliar Laboratory, where it underwent a process of air drying, followed by grinding and sieving through a 2mm mesh. Subsequently, organic matter was determined using the Walkley and Black (1934) method, while phosphorus (P) was evaluated according to the Olsen *et al* (1954) protocol, and potassium (K) was determined using the silver thiourea method. Soil analysis revealed a pH of 6.5, with a 1% organic matter content, 3.82ppm P and 295ppm K.

Soil preparation

The experimental area covered 0.16 hectares, where soil preparation was carried out to a depth of 30cm using a disc plough coupled to an agricultural tractor (New Holland, 110 HP, Model: TS6.110). To correct soil fertility, amendments such as island guano (1,450kg/ha), diammonium phosphate (150kg/ha) and potassium chloride (100kg/ha) were applied. Subsequently, plots of 6m^2 (6m $\times$ 1m) were demarcated, resulting in a total of 122 plots of one row each, corresponding to the 122 Ñuña accessions. Planting was carried out in December 2019, with an arrangement of plants at distances of 0.5m between plants and 1.0m between rows, totalling 12 plants per row, equivalent to 20,000 plants per hectare.

Table 1. Geographical origin and coding of 122 accessions of Ñuña beans from the INIA Germplasm Bank, Cajamarca, Peru.

District, Province, Region	Location	Latitude (S)	Longitude (W)	Altitude (masl)	No. of accessions	Accession code
Sanagoran, Sánchez Carrión, La Libertad	Angasmarquilla	7.72510	78.15520	3,130	9	PER002014, PER002015, PER002016, PER002017, PER002018, PER002019, PER002020, PER002021, PER002022
	Yanac	7.78020	77.95590	2,989	12	PER002023, PER002024, PER002025, PER002026, PER002027, PER002028, PER002029, PER002030, PER002031, PER002032, PER002033, PER002071
Huamachuco, Sánchez Carrión, La Libertad	Olichoco	7.81480	78.05000	3,183	10	PER002034, PER002035, PER002064, PER002065, PER002066, PER002067, PER002068, PER002036, PER002037, PER002038
Cajabamba, Cajabamba, Cajamarca	Chanshapamba	7.66750	78.05180	2,889	13	PER002039, PER002040, PER002041, PER002042, PER002043, PER002044, PER002045, PER002046, PER002047, PER002048, PER002049, PER002050, PER002051
	Chanshapampa	7.63040	78.02699	3,069	9	PER017536, PER017538, PER017548, PER017557, PER017558, PER017559, PER017571, PER017577, PER017593
	Shitabamba	7.67480	78.03730	2,821	10	PER002059, PER002060, PER002061, PER002062, PER002063, PER002072, PER017545, PER017578, PER017580, PER017583
	Churgapampa	7.65645	78.06936	2,828	1	PER017547
	Huanza	7.66693	8.07693	2,596	11	PER017537, PER017539, PER017549, PER017550, PER017553, PER017564, PER017565, PER017587, PER017588, PER017589, PER017590
	Callash	7.63970	78.06299	2,756	5	PER017540, PER017551, PER017552, PER017594, PER017595
	Cajabamba	7.62190	78.04450	2,685	2	PER002069, PER002070
	Colcabamba	7.64897	78.03058	2,899	1	PER017561
	Chanshe	7.67867	78.06359	2,660	2	PER017562, PER017563
Sitacocha, Cajabamba, Cajamarca	Lluchubamba	7.52039	77.96881	3,023	13	PER017541, PER017543, PER017568, PER017574, PER017581, PER017582, PER017591, PER017592, PER017546, PER017570, PER017573, PER017584, PER017576

Continued on next page

Table 1 continued

District, Province, Region	Location	Latitude (S)	Longitude (W)	Altitude (masl)	No. of accessions	Accession code
Condebamba, Cajabamba, Cajamarca	Cauday	7.57435	78.07074	2,815	7	PER017535, PER017556, PER017560, SC_7280, PER017572, PER017575, PER017586
	Ogosgon	7.56360	78.09330	2,697	8	PER002052, PER002053, PER002054, PER002055, PER002056, PER002057, PER002058, PER017555
	Huarasullo	7.57815	78.10208	2,588	2	PER017566, PER017567
Cachachi, Cajabamba, Cajamarca	El aliso	7.44810	78.26905	3,233	3	PER017542, PER017544, PER017554
Llacanora, Cajamarca, Cajamarca	La paccha	7.19240	78.42650	2,629	1	PER002073
Bambas, Corongo, Ancash	Bambas	8.60247	77.99639	2,931	3	PER018011, PER018012, PER018027

Cultivation treatments

Manual weeding was carried out 45 days after planting (DAP). Subsequently, trellising was carried out at 50 DAP by installing 2.5m high posts at the ends of the plots. On these posts, a galvanized wire (N° 16) was stretched along 6m, which supported the plants. A phytosanitary control against boring larvae (Lepidoptera: Noctuidae) was also implemented, using alphacypermethrin (25ml/20L of water).

Determination of variables

Evaluations were carried out from the beginning of flowering until harvest, recording data from 10 plants per accession. Descriptors adapted from the International Plant Genetic Resources Institute for *Phaseolus vulgaris* (IPGRI, 2001) were studied (Table 2). The Royal Horticultural Society Colour Chart (RHS, 2001) was used to assign the colours of flowers, pods and seeds. The study included consideration of 12 qualitative descriptors (Table 2) and 9 quantitative descriptors (Table 3).

Statistical analysis

Characterization information was subjected to descriptive and multivariate statistical analysis. Multivariate statistics included multiple correspondence analysis (MCA) for qualitative traits and principal component analysis (PCA) for quantitative traits. In both cases, decision trees were constructed using hierarchical and phylogenetic dendrograms. The Euclidean distance and the Ward.D2 (Ward, 1963) method were used as a similarity measure to carry for grouping between accessions. For the clusters of the quantitative traits, a comparison of means was carried out using Tukey's test ($p < 0.05$). The analyses were carried out with the packages Factoextra (Kassambara and Mundt, 2020) and FactorMiner (Lê *et al*, 2008) for MCA and PCA. The dendrograms were elaborated with the cluster (Maechler *et al*, 2021) and circlize (Gu *et al*, 2014) packages, while the visualization of the results was performed with ggplot2 (Wickham, 2016). Comparison of means was run with the AgroR package (Shimizu *et al*, 2023). All analyses were performed using RStudio statistical software (R Core Team, 2023).

Results

The phenotypic data for 12 qualitative and 9 quantitative descriptors collected on 122 accessions of Ñuña beans from the INIA collection are summarized in Supplemental Table 1 and were used in statistical analyses to assess and describe their genetic diversity.

Multiple correspondence analysis of qualitative characters

The qualitative characters evaluated were subjected to MCA, the results of which are presented in Figure 2. A marked association was observed between several characters, such as darker colour of seeds (DCS) and lighter colour of seeds (LCS) which presented the highest contributions to the variability of the 122 accessions of Ñuña. Seed coat pattern (SCP), flower wing colour (WIC) also associated and had similar contributions to the clustering of the accessions. There was a joint association between standard colour (STC), dry pod colour (DPC) and colour of immature pods (CIP). This pattern suggests that certain characteristics share discernible similarities, while others show a less prominent relationship. These findings provide further insight into the interrelationships between the qualitative variables assessed.

Hierarchical analysis of qualitative characteristics

The analysis of the phylogenetic hierarchical tree of the 122 accessions of Ñuña is shown in Figure 3. It is possible to observe the formation of four morphological clusters, grouped according to their most similar characters. Cluster I represents 37% of the accessions (45 accessions), which showed similarity in seven characters associated with different morphological stages: darker colour of seeds, between white-tinged black, grey-brown and greyish purple-tinged white to brown; lighter colour of seeds between white tinged purple, yellow, greyish orange, brown, purple to absent; seed coat pattern between mottled, spotted, around the hilum; standard colour between green yellow and yellow green pigmented; wing colour between white, white pigmented violet to violet blue; colour of immature pods between green, yellowish green and green pigmented; and dry pod colour between yellow and orange. Cluster II represents 4% of the accessions (five accessions), which showed similarity in nine characters associated with different phenological stages: darker colour of seeds and lighter colour of seeds dyed purple; striped seed coat pattern; purple colour of immature pods; pod curvature curved; seed shine matt; oval seed shape; intermediate leaf persistence and absent vein in seeds. Cluster III represents 7% of the accessions (nine accessions), which showed similarity in eight characters associated with different phenological stages: in the presence of a dark colour stripe moving from the hilum towards the top of the grain, the standard colour between yellow-green pigmented with purple to purple violet; the darker colour of seeds between purple with white lateral stripe and violet blue with orange-white lateral stripe; lighter colour of seeds between greyish orange with white lateral stripe to greyish purple with white lateral stripe; long-stripes seed coat pattern; wing colour between purple to purple violet; colour of immature pods between green and yellowish green; pod curvature between slightly curved to curved; dry pod colour between yellow and orange. Cluster IV represents 52% of the accessions (63 accessions), which showed similarity in seven characters associated with different phenological stages: darker colour of seeds between greyish orange, greyish red,

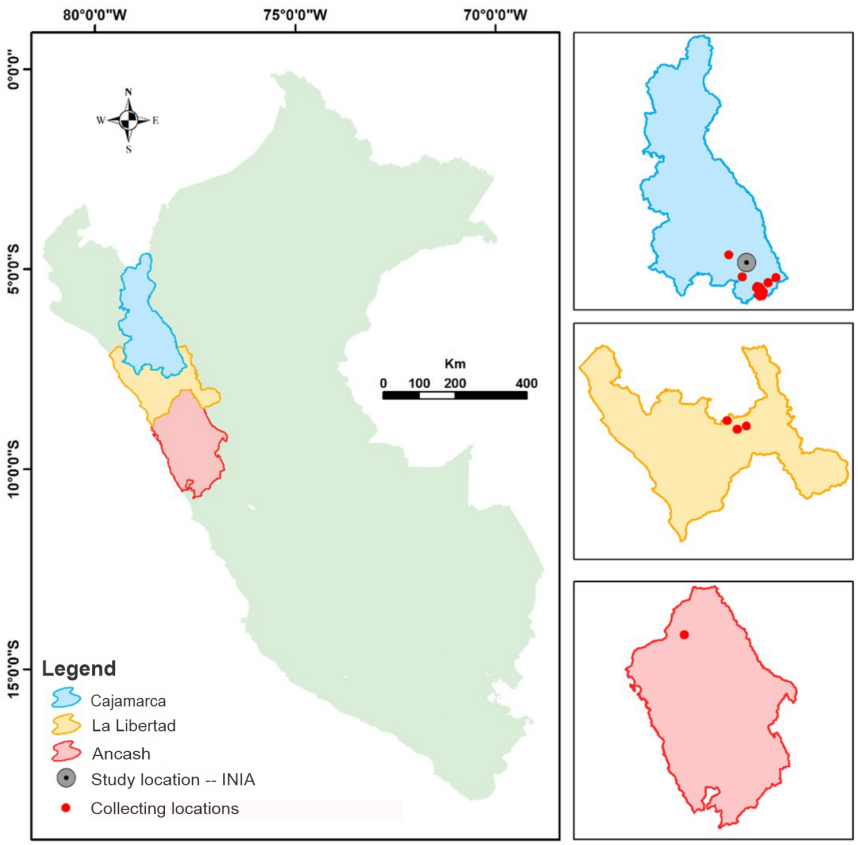

Figure 1. Map of collecting locations of 122 accessions of Ñuña beans collected in three regions in Peru and conserved in the INIA Germplasm Bank.

Table 2. Qualitative morphological characteristics assessed, codes and period of assessment. IPGRI descriptor code adapted from *Descritores de Phaseolus vulgaris* (IPGRI, 2001).

IPGRI descriptor code	Characteristic	Codes	Period of assessment
4.2.4	Standard colour	STC	Flowering
4.2.5	Wing colour	WIC	Flowering
4.2.6	Colour of immature pods	CIP	Immature pods expanded
4.2.9	Pod curvature	POC	Immature pods expanded
6.2.17	Dry pod colour	DPC	Harvest
6.1.8	Leaf persistence	LEP	When 90% of pods are dry
4.3.2	Darker colour of seeds	DCS	Grain dry
4.3.3	Lighter colour of seeds	LCS	Grain dry
4.3.1	Seed coat pattern	SCP	Grain dry
4.3.4	Seed shine	SSH	Grain dry
6.3.2	Veins in seeds	VIS	Grain dry
4.3.5	Seed shape	SES	Grain dry

grey brown, purple, violet-blue and black; lighter colour of seeds between greyish orange, brown grey, greyish purple, purple, purple or greyish red and green yellow; seed coat pattern between absent, striped, to pattern around the hilum; standard colour between purple, purple violet and yellow-green pigmented with purple violet; wing colour between white to purple violet; colour of immature pods between yellowish green and green pigmented and dry pod colour between yellow, orange and purple.

Principal component analysis (PCA) of quantitative traits

The PCA of quantitative traits explained 71.6% of the total variability and is presented in Figure 4. In this analysis, the accessions did not show differentiation of their quantitative traits according to their origin. Leaflet length and leaflet width showed a high positive correlation, as indicated by the direction of the arrows in Figure 4. The same, but to a lesser degree, occured for the characters: immature pod length, pod width, pod

Table 3. Quantitative characteristics assessed, codes and period of assessment. IPGRI descriptor code adapted from *Descritores de Phaseolus vulgaris* (IPGRI, 2001). n/a, not available in descriptor list.

IPGRI descriptor code	Characteristics	Codes	Period of assessment	Units
4.2.7	Immature pod length	IPL	Fully expanded immature pods	cm
6.2.13	Pod width	PWI	Fully expanded immature pods	mm
6.2.14	Pod beak length	PBL	Fully expanded immature pods	mm
6.3.3	Seed weight (100 units)	SEW	In dry grain, 12 to 14% moisture content	g
6.3.5.1	Seed length	SEL	In dry grain, 12 to 14% moisture content	mm
6.3.5.2	Seed width	SW	In dry grain, 12 to 14% moisture content	mm
6.3.5.3	Seed height	SEH	In dry grain, 12 to 14% moisture content	mm
4.1.1	Leaflet length	LL	At 50% flowering, on the third trifoliate leaf	cm
n/a	Leaflet width	LW	At 50% flowering, on the third trifoliate leaf	cm

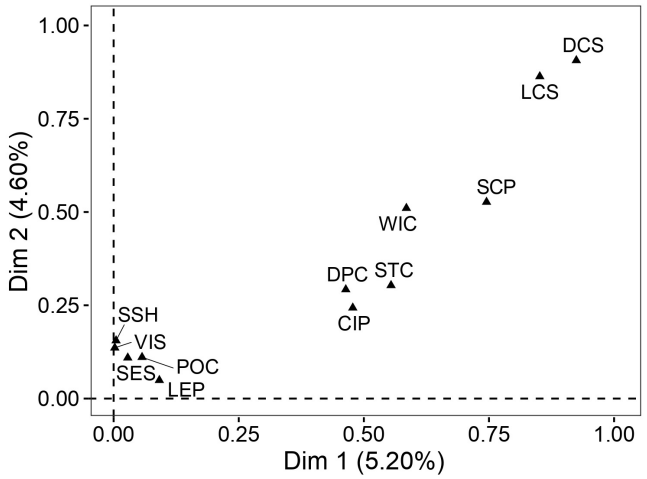

Figure 2. Multiple correspondence analysis of qualitative variables of the germplasm of Ñuña (*Phaseolus vulgaris*). DCS, darker colour of seeds; LCS, lighter colour of seeds; SCP, seed coat pattern; WIC, wing colour; STC, standard colour; DPC, dry pod colour; CIP, colour of immature pods; SSH, seed shine; VIS, seed veins; POC, pod curvature; SES, seed shape and LEP, leaf persistence.

beak length, hundred seed weight, seed length, seed width and seed height, respectively.

Hierarchical analysis of quantitative characteristics

The hierarchical cluster analysis based on the quantitative traits (Figure 5) revealed the formation of four clusters, established according to their most similar characteristics. Table 4 shows the mean values of the characters of Clusters A, B, C and D, highlighting significant differences ($p < 0.05$) between clusters. Cluster A, representing 22% of the accessions (27 in total), was characterized by lower values for immature pod length, pod width, pod beak length, hundred seed weight, seed length, seed width and seed height compared to the rest of the clusters (Table 4). Cluster B, comprising 16% of the accessions (20 in total), was significantly superior to the other clusters showing higher values for immature pod length, pod width, pod beak length, hundred seed weight, seed length, seed width and seed height. However, Cluster B had lower values for leaflet length and leaflet width compared to the other clusters. Cluster C, comprising 30% of the accessions (37 in total), showed higher mean values than Cluster A, but lower than Clusters B and D for the character's immature pod length, pod beak length, hundred seed weight, seed length, seed width and seed height. For width of immature pod trait, Cluster C had a higher mean than Clusters A and D, but lower than Cluster B. Cluster C had a lower mean for the leaflet length trait, than the rest of the clusters. For the leaflet width character, the mean of Cluster C was lower than Clusters A and D, but higher than the mean of Cluster B. Finally, Cluster D, comprising 31% of the accessions (38 in total), was characterized by higher values than Clusters A and C for immature pod length, pod beak length, hundred seed weight, seed length, seed width and seed height. As for pod width, its mean value was higher than that of Cluster A, but lower than the means of Clusters B and C. Cluster D stood out with higher values for leaflet length and leaflet width compared to Clusters A, B and C. There was a marked diversity in the distances between clusters, particularly between Cluster A and Clusters C and D. This suggests that the accessions in these clusters show significant morphological differences.

Discussion

Qualitative characterization

Qualitative analysis revealed that characters associated with seed colour showed the greatest morphological variability, followed by seed coat pattern and, to a lesser degree, flower-related characters (Figure 2). This indicates that seed-related characters are fundamental for the identification and differentiation of Ñuña bean accessions, establishing associations between seed morphology and the phenotypic diversity observed among them. This result is consistent with previous studies by Martirena-Ramírez *et al* (2017) and Espinosa-Pérez *et al* (2015), who highlighted that flower colour and seed colour are essential to assessing phenotypic variability of common bean. Similarly, Morales-Morales *et al* (2019) have determined the same for cowpea

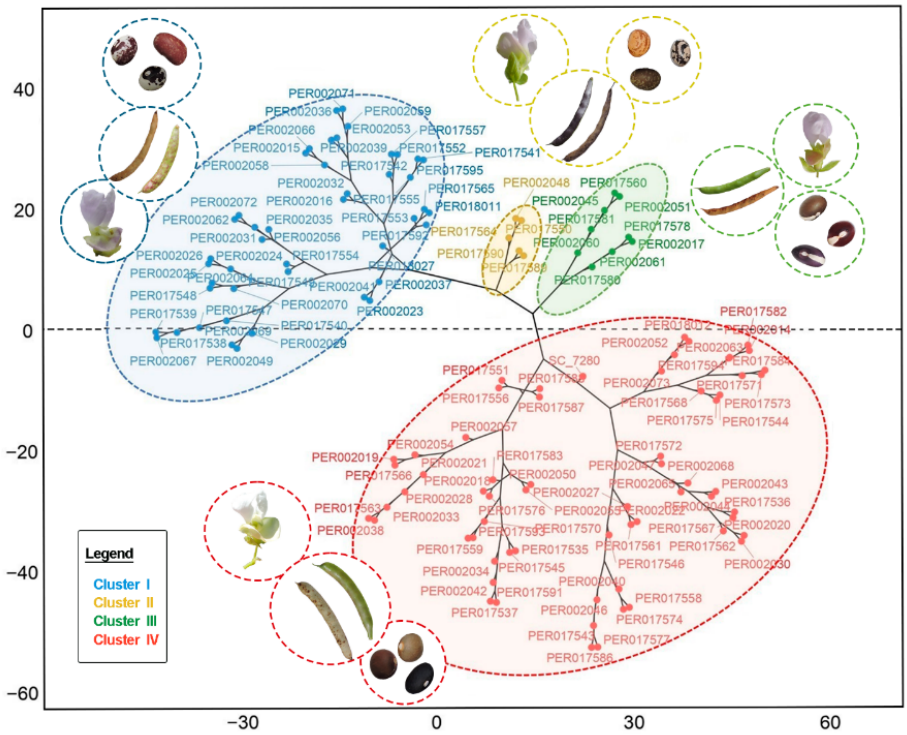

Figure 3. Hierarchical clustering based on multiple correspondence analysis of qualitative variables of the germplasm of Ñuña (*Phaseolus vulgaris*). DCS, darker colour of seeds; LCS, lighter colour of seeds; SCP, seed coat pattern; WIC, wing colour; STC, standard colour; DPC, dry pod colour; CIP, colour of immature pods; SSH, seed shine; VIS, veins in seeds; POC, pod curvature; SES, seed shape and LEP, leaf persistence. The photographs inserted next to each group show the representative qualitative traits of each group.

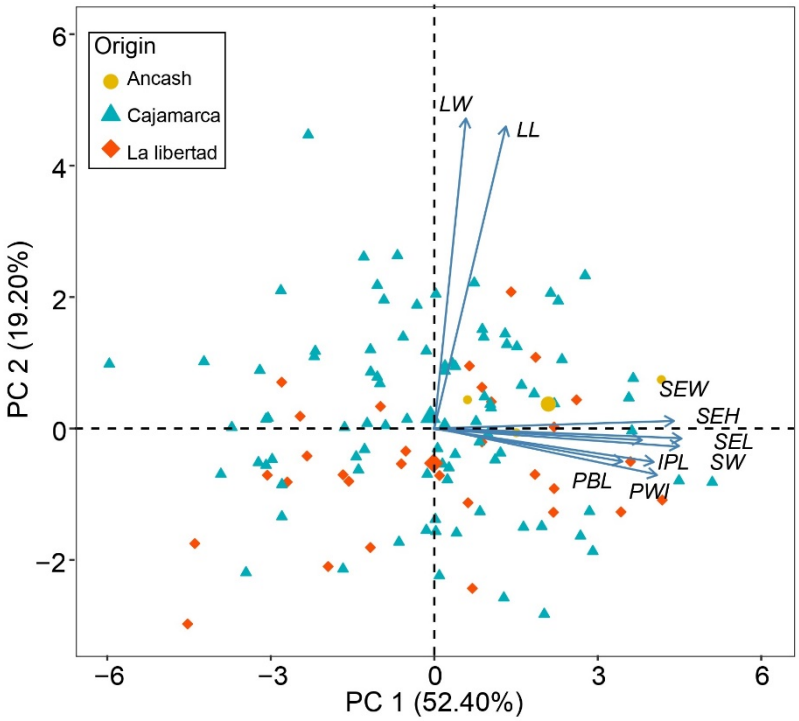

Figure 4. Projection of nine quantitative characters in the first two dimensions of the Principal Component Analysis of 122 Ñuña (*Phaseolus vulgaris*) accessions from the germplasm collection of INIA, Estación Experimental Agraria Baños del Inca, Cajamarca, Peru. Accessions are colour coded by origin regions. The arrows indicate the inertia of the contribution of the characters IPL, immature pod length; PWI, pod width; PBL, pod beak length; SEW, seed weight; SEL, seed length; SW, seed width; SEH, seed height; LL, leaflet length; and LW, leaflet width. And the distance between them implies their correlation.

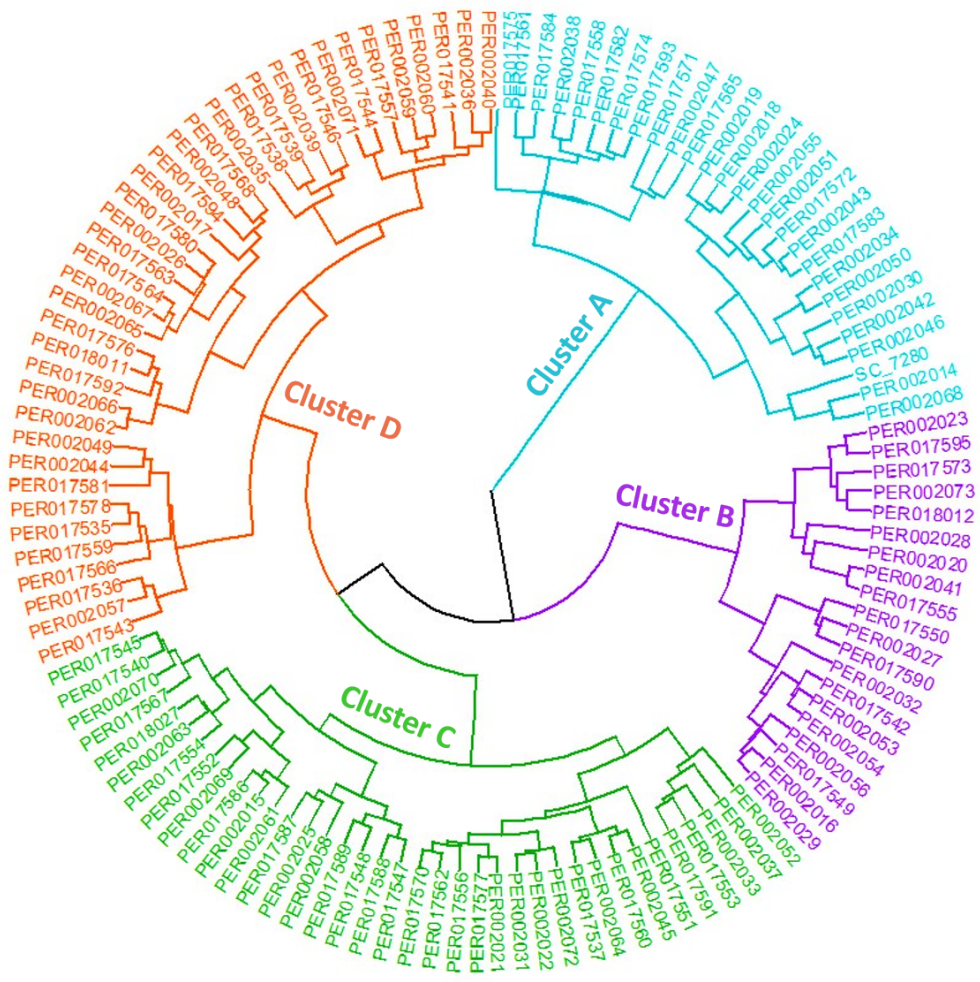

Figure 5. Hierarchical dendrogram of Ñuña (*Phaseolus vulgaris*), Ward.D2 method. Euclidean distance based on nine quantitative characters from the germplasm collection of INIA, Estación Experimental Agraria Baños del Inca, Cajamarca, Peru.

Table 4. Descriptive analysis and comparison of means between clusters for quantitative traits. CV, coefficient of variation; MSD, minimum significant difference; IPL, immature pod length; PWI, pod width; PBL, pod beak length; SEW, seed weight; SEL, seed length; SW, seed width; SEH, seed height; LL, leaflet length; and LW, leaflet width. *Means followed by the same letter in the rows do not differ statistically from each other, according to Tukey's test ($p < 0.05$).

Character	Average character values *				MSD	CV(%)
	Cluster A	Cluster B	Cluster C	Cluster D		
IPL (cm)	10.38 c	13.88 a	11.59 b	12.34 b	0.77949	9.46
PWI (mm)	12.55 c	15.35 a	14.59 b	14.29 b	0.58016	5.93
PBL (mm)	9.34 d	12.55 a	10.59 c	11.55 b	0.91175	12.08
SEW (g)	41.10 c	73.43 a	55.85 b	60.08 b	6.18127	15.77
SEL (mm)	8.99 d	13.40 a	10.24 c	11.53 b	0.74487	9.91
SW (mm)	7.50 c	9.10 a	8.35 b	8.56 b	0.27129	4.71
SEH (mm)	6.76 c	7.97 a	7.45 b	7.55 b	0.27629	5.40
LL (cm)	11.82 b	11.75 b	11.67 b	13.66 a	0.93886	11.02
LW (cm)	8.48 b	7.90 b	8.15 b	9.73 a	0.68477	11.43

(*Vigna unguiculata* L. Walp). Murga-Orrillo *et al* (2024) affirmed that in cowpea seed colours mark the preferences of producers and consumers since the dark colour of the testa presents greater antioxidant activity. From this perspective, a generalized tendency in bean research to use morphological characters of the seed as primary indicators of genetic variability is confirmed, underlining its importance in the classification and differentiation of accessions.

The phylogenetic tree presented in Figure 3 showed that the qualitative characters related to seed, flower and pod are valid phenotypic traits to discriminate morphologically the different accessions of Ñuña; these characteristics, which vary among accessions, could be related to their areas of origin. In addition, it highlighted that there were no duplicate accessions in the studied collection at the phenotypic level. These results are valid for genetic diversity conservation since they indicate that the 122 accessions could represent unique genetic resources, which should be studied in detail to determine the variations of these accessions due to environmental and anthropogenic factors. Also, Cruz-Balarezo *et al* (2009) conducted clustering analyses on 24 accessions of Ñuña, which determined that there was no duplication of germplasm, even in cases where the accessions shared similar characters. On the other hand, in common bean, Vásquez *et al* (2024) identified four distinct morphological groups in 58 accessions with promising characteristics for breeding programmes. These studies highlighted the importance of characterizing Ñuña bean accessions for conservation and breeding initiatives.

Quantitative characterization

Regarding the spatial distribution of the 122 Ñuña accessions, based on quantitative traits, no defined clustering pattern for geographic origin was identified (Figure 4). This could be due, in part, to similar climatic conditions in the Andean region or common agricultural practices (Figure 1). Mishra *et al* (2010) have shown that genetic diversity among bean genotypes did not show a direct relationship between clustering patterns and geographic origin. Consequently, the maintenance of Ñuña bean genetic characteristics from different geographic origins is crucial to preserving its biodiversity and genetic improvement. The PCA presented in Figure 4 shows strong positive correlations between leaf, immature pod and seed traits, demonstrating that these traits are mutually dependent. This could be related to shared developmental processes or the expression of common genes that regulate the size and structure of the mentioned plant organs. In common bean, Lescay-Batista *et al* (2017) and García-Fernández *et al* (2023) found a close association between pod characters and seed weight. Also, in lima bean (*Phaseolus lunatus* L.), a strong correlation was found between yield and stem length, number of seeds per pod, hundred-seed weight, primary leaf length and width, pod weight and pod length (Akande and Balogun, 2007; López-Alcocer *et al*, 2016).

The four clusters identified in Figure 5 were evaluated through a mean comparison test ($p < 0.05$), as detailed in Table 4. Cluster B presents significant differences, with higher means in the traits related to productivity, compared to the means of Clusters A, C and D. The accessions of Cluster B will be valuable in genetic improvement programmes because they could provide higher yields. Also, Pesantes-Vera and Soto (2013) in Ñuña, and Kinhoégbè *et al* (2020) in pigeonpea (*Cajanus cajan* (L.) Huth), used characters related to productive yield in the selection of promising individuals. However, it is essential to conduct further studies on the 122 accessions of Ñuña to identify relevant traits, such as resistance to abiotic and biotic factors, as well as nutritional aspects, in order to enrich future breeding programmes.

Conclusions

In the qualitative traits of Ñuña, MCA determined that the most significant contributions to the variability of the accessions were the seed heads' dark and light colours. In the phylogenetic hierarchical analysis, four clusters were formed, represented by 37% (I), 4% (II), 7% (III) and 52% (IV) of the accessions respectively.

In the quantitative characters of Ñuña, PCA showed no discrimination between regions of origin of the accessions, but a high positive correlation between length and width of leaves, as well as between length and width of pods, and length, width, height and weight of seeds.

In the hierarchical analysis of the quantitative characters, four clusters were also formed, represented by 22% (A), 16% (B), 30% (C) and 31% (D) of the accessions. Subjected to comparative analysis of means, these clusters showed significant differences ($p < 0.05$) with higher values in cluster B for pod length and width, length, width, height and seed weight.

Understanding the variability of qualitative and quantitative traits of Ñuña, and classifying them, is the starting point for subsequent genetic improvement studies aimed at obtaining Ñuña cultivars with better yields and high nutritional value, resistant to biotic and abiotic factors.

Authors contribution

Angel Esteban Santa Cruz Padilla: conceptualization, formal analysis, writing – original, research, data curation, resources, methodology, proofreading and editing.

Jorge Luis Vásquez-Orrillo: conceptualization, formal analysis, writing – original, research, data curation, resources, methodology, proofreading and editing.

Ricardo Manuel Bardales-Lozano: formal analysis, writing – original, research, methodology, proofreading and editing.

Hipolito Murga-Orrillo: formal analysis, writing – original, research, methodology, proofreading and editing.

Conflict of interest statement

The authors have declared that no competing interests exist.

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Supplemental data

Supplemental Table 1. Agromorphological characterization data of 122 Ñuña accessions from the INIA Germplasm Bank - Peru.

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Survey on threatened medicinal plants diversity of Northwestern Syria

Munzer Aldarvish *, **Anas Al Kaddour**[a], **Akram Bourgol, Yasser Ramazan, Yousef Hallak**[b], **Stephen Cavers**[c] **and Joan Cottrell**[d]

[a] *General Organization for Seed Multiplication, Syria*
[b] *Ministry of Agriculture, Syria*
[c] *UK Centre for Ecology & Hydrology, Bush Estate, Penicuik, Midlothian, EH26 0QB, UK*
[d] *Forest Research, Northern Research Station, Roslin, Midlothian, UK*

Abstract: Throughout history, many plant species have been used as natural medicines to prevent and treat human diseases. Due to its geographical location, climate, and history, Syria contains a remarkable diversity of medicinal plants. However, in recent years a prolonged period of conflict has resulted in widespread ecosystem destruction, human population displacement, and disruption of farming practices. Although poorly documented this is believed to have resulted in a significant decline in medicinal plant populations.

In this study, we used structured interviews with local agricultural experts to collect basic information on the current status and critical threats to medicinal plant species in northwest Syria. Our results show that many of these species have experienced genetic erosion and deterioration due to a combination of overuse (massive unmanaged gathering of medicinal plant material) and climatic changes, particularly those relating to more frequent droughts. To initiate *ex situ* conservation initiatives, the locations of medicinal plants exposed to deterioration were identified from the results of a questionnaire. Seeds from seven species: chamomile (*Matricaria chamomilla* L.), wild thyme (*Thymus capitatus* L.), sage (*Salvia officinalis* L.), hyssop (*Hyssopus officinalis* L.), caper (*Capparis spinosa* L.), basil (*Ocimum basilicum* L.), and watercress (*Nasturtium officinale* R. BR.) were collected for the establishment of *ex situ* collections in the future. We discuss the potential for recovery initiatives to protect and conserve these species and to support the sustainable use of medicinal plant genetic resources in Northern Syria. Such endeavours are vital for the continued well-being of the Syrian population and humanity as a whole.

Keywords: plant genetic resources, medicinal plants, genetic erosion, *in situ* conservation, *ex situ* conservation

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Introduction

Medicinal and aromatic plants represent valuable global resources for the practice of traditional medicine as well as the development of novel pharmaceuticals (Nalawade *et al*, 2003; Hamilton, 2004; Chacko *et al*, 2010; Chen *et al*, 2010; Alachkar *et al*, 2011; Asiimwe *et al*, 2021; Pakdemirli *et al*, 2021). Several studies showed that there is a strong and continuing scientific and commercial interest in documenting the traditional uses of medicinal plants collected from their natural habitats and in exploring new applications for them (Daily, 1997; Ecological Society of America, 1997; Nasrallah *et al*, 2020). The heightened demand for herbal medicines, natural health products and secondary compounds from medicinal plants is rapidly reshaping their use worldwide (Saxena *et al*, 2007; Khatib *et al*, 2021).

In many regions of the world, the medicinal plant market is intimately connected with the livelihoods of people and their primary healthcare (Saxena *et al*, 2007; Valderrabano *et al*, 2018). Collecting medicinal

*Corresponding author: Munzer Aldarvish (drgeneral2015@yahoo.com)

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plants and plant materials from the wild contributes to the subsistence livelihoods of many Indigenous People. Even today, it is estimated that hundreds of millions of people, primarily in developing countries, derive a significant part of their sustenance and income from collecting plant and animal products**.** Furthermore, a remarkable 70% of the global human population relies in some way on medicinal plants for their healthcare needs, underscoring the pivotal role these plants play in the economies of biodiversity-rich countries, through export and import (Walter, 2001). In addition to the collection from wild populations, people cultivate various medicinal plants in their home gardens for everyday use, as their access to modern medicines and healthcare facilities is often limited (Al-Oudat and Laham, 1994; Agelet *et al*, 2000; Kandari *et al*, 2012)

The surge in demand for medicinal plants in urban areas and high unemployment rates in rural areas means a much wider range of people are now accessing wild medicinal plant resources beyond the restricted set of specialists who were previously dominant in this role (Nahashon, 2013).

As well as direct pressure from harvesting, many medicinal plants are also under pressure from bio-prospecting – collection in pursuit of sources of new biochemicals. Such unregulated commercialization may lead to these vital plant resources becoming inaccessible and unaffordable for populations that have relied on them for centuries, as well as denying them to people elsewhere in the world (Roberson, 2008). Furthermore, the profits from such commercial exploitation are rarely returned to the people who may have provided Indigenous knowledge, neglecting the access and benefit-sharing aspects of the Nagoya Protocol (Knight *et al*, 2023). Finally, medicin**a**l plant species also face general threats from habitat loss and climate change. As wild areas are destroyed or degraded, unique and valuable species are lost along with the habitat. This loss of biodiversity may also result in the loss of essential remedies for current and future diseases. Due to all these pressures, medicinal plant populations are disappearing at an alarming rate, and conservation measures are urgently needed.

There are well-established methods for the conservation and sustainable use of medicinal plant resources. Rajasekharan and Shabir (2020) assert that both conservation (*in situ* and *ex situ* conservation and cultivation practices) and resource management strategies (e.g. good agricultural practices and sustainable use solutions) should be developed in parallel and adhered to, in order to secure their long-term future. However, despite the availability of guidance, only a small fraction of medicinal plant species are being protected through traditional conservation methods in nature reserves or botanical gardens (Chen *et al*, 2016). Most collection practices for medicinal plants are unsustainable, with impacts including overharvesting, lack of replenishment in the wild, and poor cultivation practices. Consequently, many species are now endangered or at risk of extinction and several have recently been included on the IUCN Red List (Walter, 2001; Baričevič *et al*, 2002; Nahashon, 2013; Rajasekharan and Shabir, 2020)

Although extinction is the most serious impact on society, the loss of even a proportion of a species impacts diversity and translates into the loss of potential new medicinal discoveries (Kumar, 2006).

Syria, situated within a region characterized by substantial topographical and climatic diversity in the area known as the Fertile Crescent, possesses a remarkable abundance of agro-biodiversity. Of particular note, it has a rich diversity of plants used by local communities for medicinal purposes. The use of Indigenous plants is widespread at the community and end-user levels (Khatib *et al*, 2021). A study by Kywan (2016) highlighted that among the 394 cultivated plant species grown in Syria, 91 were classed as medicinal plants and were either collected from the wild or grown in home gardens. Medicinal, aromatic wild plants represent a large part of Syria's flora. Various plants, including *Matricaria* sp., *Thymus* sp., *Artemisia* sp. are gathered directly from the wild by rural communities for use in traditional medicine (FAO, 2001). Traditional medicine is a significant healthcare system in Syria, where people rely on various natural substances as sources of treatment (Alamholo *et al*, 2023). These plants are integral to traditional medicine, especially in impoverished regions. At present, documentation of Syria's agro-biodiversity is inadequate, but what little information is available indicates the presence of numerous globally significant species. Disturbingly, there have been documented declines in several species in Syria, especially wheat, along with both cultivated and wild barley, as well as various types of legumes and vegetables – whether domesticated or wild – are at risk of degradation and extinction (FAO & ITPGRFA, 2022). Also, Handa *et al* (2006) noted that Syria's mild climate is ideal for growing a variety of plants. Many of these plants are used in Syrian culture for aromatherapy, perfumes and medicine. Syria is home to approximately 3,459 plant species, spread across 865 genera and 131 families (FAO, 2001). A significant number of these species are valued for their medicinal and aromatic properties. Rural communities often gather these plants from the wild to prepare traditional medicines. However, Syria's medicinal and aromatic plants face threats from issues such as forest destruction due to fires, overgrazing, urban expansion, water scarcity, tree cutting for fuel and unsustainable harvesting (FAO & ITPGRFA, 2022). To safeguard these plants, there is a need for improved management, conservation, research into traditional knowledge and medicine, and the regulation of herbal medicine production and trade.

Within Syria, a range of pressures are threatening the medicinal flora, including a changing climate (drought, rising temperature), wildfires, overgrazing, uncontrolled urban expansion, internal displacement, and extensive overharvesting. In particular, the expansion of agricultural cultivation into new areas, and the

adoption of economically significant crops like cereals, forages and legumes, are driving substantial habitat loss (Syrian Government, UNDP, GEF, 2009; Al-Darvish *et al*, 2022). Although the deterioration of plant genetic resources in Syria had begun before the ongoing political crisis started (FAO, 1996), it has amplified existing threats and it will be imperative to quantify the scale of this effect (Kywan, 2016; Gaafar, 2021; Geoglam Crop Monitor, 2021). Alongside direct effects, such as armed conflicts in mountainous regions involving landmines and explosives, the civil war has also severely impaired the research and regulatory infrastructure, with institutions formerly dedicated to conserving and propagating genetic resources for future generations now closed. There are currently no conservation activities for medicinal plants taking place, not least because Syrian botanists have been moved or diverted to other activities. The loss of competent authorities, along with any effective government oversight, has undermined a national process based on Key Biodiversity Areas (KBAs) and now management and conservation plans that address both ecological and social dimensions are urgently needed, to avoid extinction and promote restoration and sustainable use practices (Valderrabano *et al*, 2018).

As a first step, there is an urgent need to assess the current status of medicinal plant populations and identify cost-effective strategies for conserving those most at risk of genetic erosion and degradation (Valderrabano *et al*, 2018). The research reported here was designed in accordance with the Syrian National Strategy for Conservation and Management of Plant Genetic Resources for Food and Agriculture 2015–2035 (FAO, 2015), and aimed to:

- Collect information about the current status and most pressing threats to medicinal plants in Northern Syria, using a questionnaire
- Characterize and map the distributions of medicinal plant species facing deterioration
- Collect seeds from multiple genotypes of each of the seven medicinal plant species for the future establishment of an *in situ* and *ex situ* collection.

Materials and methods

The study was conducted from January to September 2023 across specific subdistricts in the Idlib Governorate (Harim, Mhambal, and Darkosh) and the Aleppo Governorate (Jebel Saman and Atareb) of Northern Syria (Figure 1). These locations were identified after a series of focus group discussions (FGDs) with local communities and stakeholders in Northern Syria, including agricultural experts, community leaders and senior farmers. Representatives in each subdistrict nominated key informants (KIs), allowing for the targeted selection of knowledgeable individuals with local expertise. The FGDs revealed that these subdistricts were particularly exposed to ecosystem deterioration and disruption due to prolonged conflict, making them priority sites for assessing the status and threats facing medicinal plant species.

Data collection aimed to evaluate the current status and conservation needs of locally valuable medicinal plant species in these ecologically vulnerable areas. A questionnaire (Supplemental Data) was developed and administered to 50 KIs between 1 April 2023 and 1 August 2023. The questionnaire was designed with diverse question types to gather detailed information. Participants mainly chose answers from a predefined list, ensuring consistency across responses, but also had the option to add any additional answers not included in the predefined list. KIs, nominated by subdistrict representatives, included senior farmers, agricultural engineers and researchers, chosen for their expertise and involvement with local agricultural practices. The participant composition consisted of 76% male and 24% female respondents aged 24–85, with 36% agricultural researchers, 34% agricultural engineers and 30% senior farmers, offering a comprehensive perspective on the perceived threats to the selected species and potential conservation interventions.

Survey and data analysis

The survey aimed to capture respondents' perceptions of critical threats to medicinal plant species – chamomile (*Matricaria chamomilla* L.), wild thyme (*Thymus capitatus* L.), sage (*Salvia officinalis* L.), hyssop (*Hyssopus officinalis* L.) and caper (*Capparis spinosa* L.), which were considered by local experts to be the species most vulnerable to deterioration due to diverse factors such as overharvesting, unmanaged collection, and climate-driven droughts. Collected data were analyzed in MS Excel (Microsoft Office 2020) to assess the prevalence and distribution of responses for each question. The analysis included calculating percentages, frequency counts, and averages, providing a comprehensive view of response patterns, and highlighting key trends across participants' answers.

Ex situ conservation methodology

For *ex situ* conservation, seeds from five mature individual plant genotypes (1,000 seeds of each genotype) of each identified species were collected within each subdistrict, ensuring representation across ecological variations in Northern Syria. The seeds were harvested, dried and disinfected using a thiram fungicide.

For proper tracking and future use, each seed sample was labelled with essential 'passport' information, including the species name, original collection location, and collection date, which includes the date the seeds were placed in storage. The seeds were then stored in paper bags, which were placed within airtight plastic containers along with dry silica gel to maintain optimal humidity levels at ambient room temperature (approximately 20–25°C). Seed samples have been temporarily stored in a designated room at the field staff's home. .

Results

Data collected from 50 key informants, including farmers, agricultural engineers and researchers in five t arget r egions w ithin t he A leppo a nd Idlib Governorates in Northwestern Syria are detailed in Supplemental Table. The results indicated that seven species mentioned in this study showed signs of deterioration across the locations surveyed (Figure 1); the seven species showing this deterioration are given in Table 1.

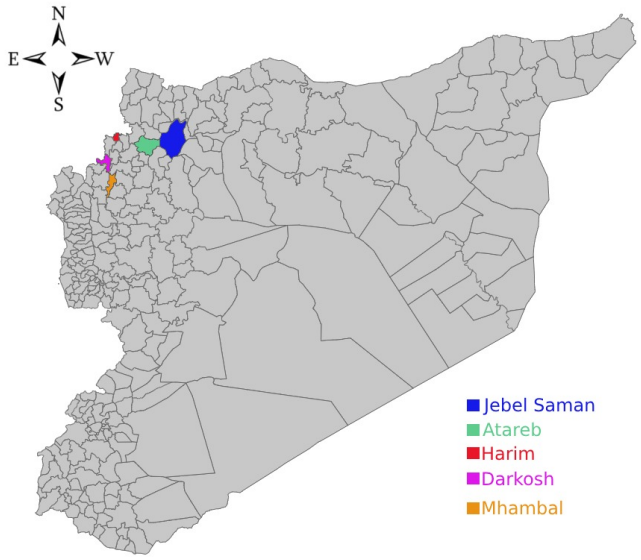

Figure 1. Map of Syria showing the study area (Subdistricts within the Aleppo and Idlib Governorates) in the north of the country. Source: Humanitarian Data Exchange

Across the study area, most (98%) of participants stated that wild thyme was susceptible to degradation, followed by chamomile (94%), hyssop (76%), sage (76 %), basil (42%), watercress (38%) and caper (14%) (Figure 2).

Responses to the questionnaire (Supplemental Table) indicated that the species considered to be most in decline varied across the five s ubdistricts. M ost of the respondents in Mhambal (86%) mentioned that Caper was in decline, whereas those who mentioned the decline of chamomile (17–21%) and wild thyme (18–20%) were more evenly distributed across the five subdistricts (Figure 3). Although seven species were listed as being in decline in the study region, they were not in decline across all subdistricts. For example, none of the respondents from Harim listed sage or hyssop as being in decline and none of the responders from Jebel Saman mentioned that caper and watercress were in decline.

For all species except wild thyme, all respondents considered each species to be 'rarely available'. For wild thyme, 98% of respondents considered it to be 'rarely available', and 2% considered it to be 'not available'.

There was complete agreement among respondents regarding the natural habitat of the seven species. Wild thyme, sage and hyssop were considered to grow in natural public spaces and forested areas. In contrast, caper and chamomile were considered to occur along agricultural roadsides, while watercress grew near freshwater streams. Basil, on the other hand, is typically cultivated at home.

Respondents were asked to assess the importance of a list of reasons for the decline of medicinal plant species in Northern Syria. The results show that respondents considered the primary factor contributing to the deterioration of these species to be excessive use, characterized by unmanaged gathering of plant material, with 47% of respondents considering this to be a contributory factor. In addition, frequent droughts, climatic changes, desertification and the loss of forested areas to urban expansion were also identified as significant factors by a large number of respondents. To a lesser extent, respondents considered neglect by local authorities, lack of awareness regarding the importance and value of these species, and the repercussions of the Syrian crisis, notably the lack of law enforcement, to play a role. The lack of interest and understanding in the use of medicinal plants among the younger generation and overgrazing were also noted by some as contributory factors but were only mentioned by 5% of the respondents. Interestingly, deforestation and floods were not considered by any of the respondents to have a discernible impact on the deterioration of these species.

When asked specifically about the effects of the Syrian crisis in exacerbating the deterioration of these medicinal plant species, 60% of respondents indicated that the crisis had a substantial role. This was primarily attributed to the absence of both interest and legal measures aimed at safeguarding and preserving these botanical species. In contrast, 40% of participants expressed uncertainty regarding the impact of the crisis.

It was notable that all participants stated that they continue to be interested in cultivating some of these species, with 66.7% stating that these species are for family use and 33.3% stating that these species are of economic use.

According to respondents, the local population in Northern Syria uses these medicinal plant species to treat a range of complaints and diseases (Table 2), with some complaints being treated by several species. For example, respiratory diseases were treated by chamomile, wild thyme, hyssop and basil. Others were only treated by one species. For example, blood pressure is only treated with basil, and anorexia is only treated with sage. Consequently, the loss of a certain species might mean that there is no other medicinal plant available to replace it as a treatment.

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Table 1. Medicinal plant species identified by the key informants as most vulnerable to genetic erosion and deterioration.

English name	Family	Scientific name	Life cycle
Basil	Lamiaceae	*Ocimum basilicum* L.	Annual
Caper	Capparaceae	*Capparis spinosa* L.	Perennial
Chamomile	Asteraceae	*Matricaria chamomilla* L.	Annual
Hyssop	Lamiaceae	*Hyssopus officinalis* L.	Perennial
Sage	Lamiaceae	*Salvia officinalis* L.	Perennial
Watercress	Brassicaceae	*Nasturtium officinale* R. Br.	Annual
Wild thyme	Lamiaceae	*Thymus capitatus* L.	Perennial

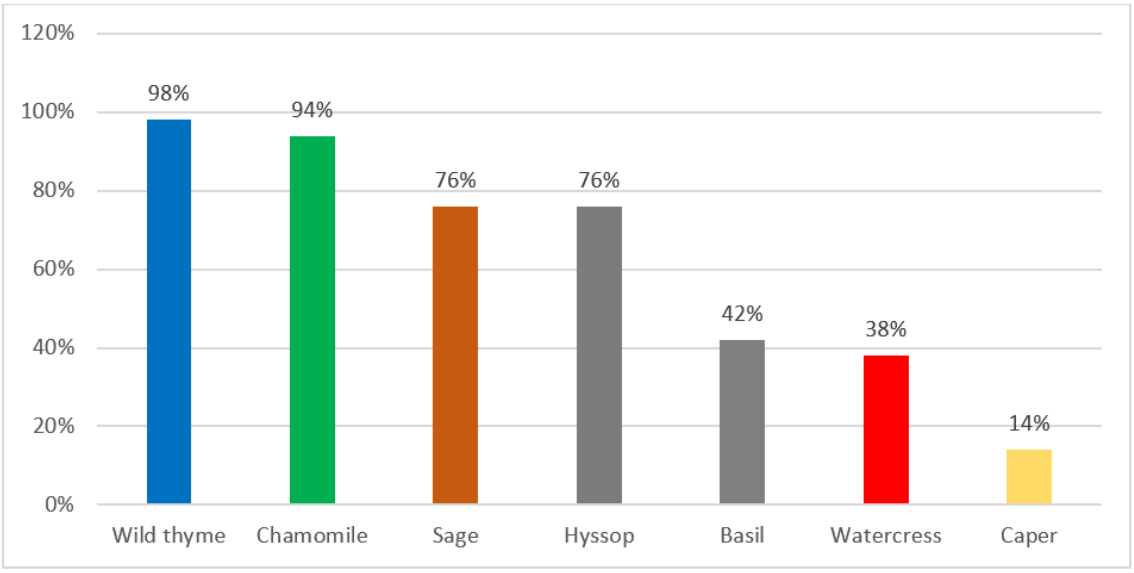

Figure 2. The percentage of respondents who listed each medicinal plant species as being in decline in Northern Syria (N=50).

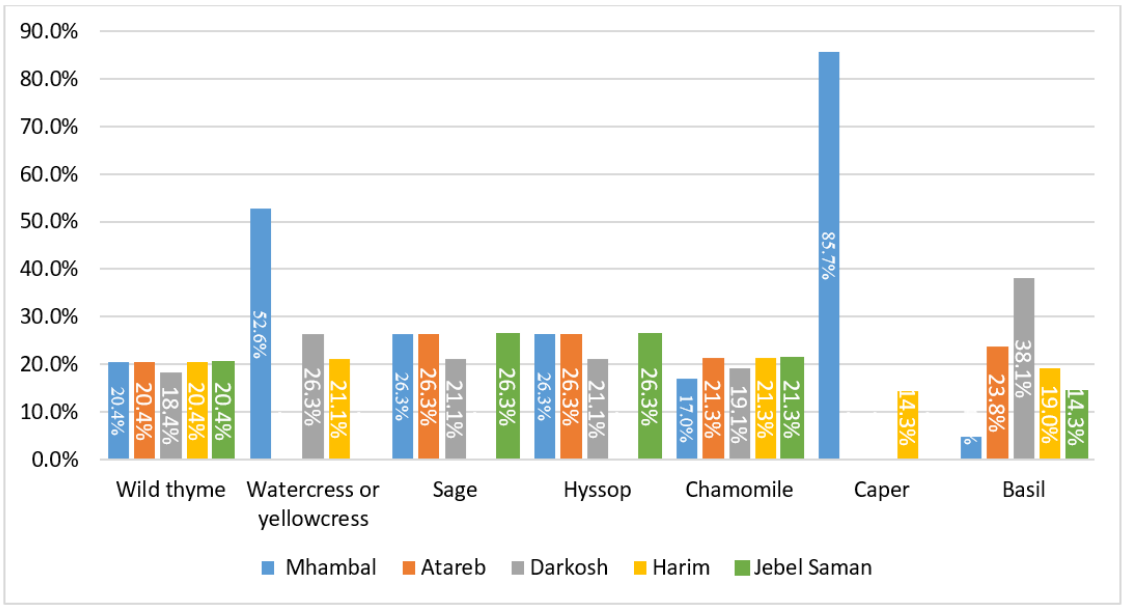

Figure 3. Proportions of all respondents who listed a particular species as exposed to deterioration (100%) according to subdistrict (N=50).

Table 2. List of diseases and complaints for which the seven medicinal species are used as treatment locally in Northern Syria, according to survey respondents.

Species	Use in traditional medicine
Chamomile	Infections of mouth and gums, respiratory diseases, colds, headaches; calming the nerves; protecting skin; regulating blood sugar; strengthening immune system
Wild thyme	Infections and inflammations of the digestive system, respiratory problems, coughs, worms; antispasmodic
Sage	Anorexia, flatulence, stomach pain or inflammation, diarrhoea, colic, stomach ulcers, indigestion
Hyssop	Respiratory diseases
Caper	Diabetes, skin diseases, anaemia, strengthening immunity, promoting weight loss
Basil	Enhance the functioning of the digestive system, cleanse the intestines, prevent constipation, relieve pain, enhance the health of the heart and arteries, treat colds and asthma, treat respiratory infections, and treat anaemia
Watercress or yellowcress	High blood pressure, increases sexual energy in men, diuretics, anaemia, expectorant

with sage. Consequently, the loss of a certain species might mean that there is no other medicinal plant available to replace it as a treatment. To guarantee the availability of seeds from these varieties, *ex situ* conservation was employed in this study. Seeds from five plant genotypes were collected, dried, disinfected, and placed in paper bags within airtight containers at ambient temperature. This approach allowed for accurate tracking of the samples, with vital passport information documented for future reference. The seeds are currently stored temporarily to preserve their viability until more sustainable long-term storage solutions can be adopted

The data from the questionnaire revealed that every respondent displayed a strong commitment to safeguarding medicinal plant species. The results highlighted that the most popular method for protecting these seeds was seed collection and conservation of these species in the local gene bank. Followed by raising awareness about the need to safeguard these species, as well as the implementation of practical protective measures in the areas where these species are found to counteract the factors leading to their decline. The idea of the establishment of local collections received significant support. In contrast, seed exchange was the least favoured method among respondents (Figure 4).

Discussion

Syria, located in the Fertile Crescent, a region known for its significant topographical and climatic variety, boasts a rich diversity of plants used by local communities for medicinal purposes. These plants play a crucial role in plant diversity and contribute to global genetic resources, as highlighted by various studies (Chacko *et al*, 2010; Chen *et al*, 2010; Alachkar *et al*, 2011; Asiimwe *et al*, 2021; Pakdemirli *et al*, 2021). However, the ongoing armed conflict has led to the overexploitation of local resources, including the excessive harvesting of medicinal plants. The absence of regulatory entities, protective laws and sustainable management practices leaves these resources unprotected, threatening their survival and regeneration. On top of this, forest fires and overgrazing have caused increased deterioration of plant populations in the region (Handa *et al*, 2006; Valderrabano *et al*, 2018; Gaafar, 2021; Khatib *et al*, 2021; Al-Darvish *et al*, 2022, 2023). This is a worldwide phenomenon, with loss of wild genotypes having been reported by several studies (Walter, 2001; Baričevič *et al*, 2002; Kumar, 2006; Roberson, 2008; Nahashon, 2013; Chen *et al*, 2016; Rajasekharan and Shabir, 2020; Geoglam Crop Monitor, 2021) but the issue is particularly acute in Syria.

Medicinal plant species, which have been used by humans for thousands of years, are a key component of traditional medicine practices worldwide. In Syria, rural communities gather medicinal plants from the wild for use in traditional healing (FAO, 2001; Alamholo *et al*, 2023). These plants have gained increasing significance with the rise of folk healers who offer treatments with fewer side effects compared to synthetic drugs. Medicinal plants also have economic value, as they can serve as a source of raw materials for the pharmaceutical industry. However, in Northern Syria, medicinal plant species are facing a decline. Our questionnaire results have provided valuable insights into the current status of these plants and the perceived causes of their deterioration.

According to data collected from a sample of 50 expert respondents, seven medicinal plant species in Northern Syria were identified as experiencing deterioration. These species are used to treat at least 24 different medical conditions, meaning their loss would significantly impact the local population, who often rely on these plants as their primary form of medical treatment. Some conditions can be addressed by multiple species, while others depend on a single species for treatment. The loss of plants with unique medicinal properties would be especially detrimental to public health, particularly if no alternative plant species with similar properties are available.

Chamomile stands out among these species due to its broad medicinal applications for various complaints. It was one of the seven species identified as deteriorating across all five subdistricts. Given its wide usage and the decline it is facing, chamomile warrants priority

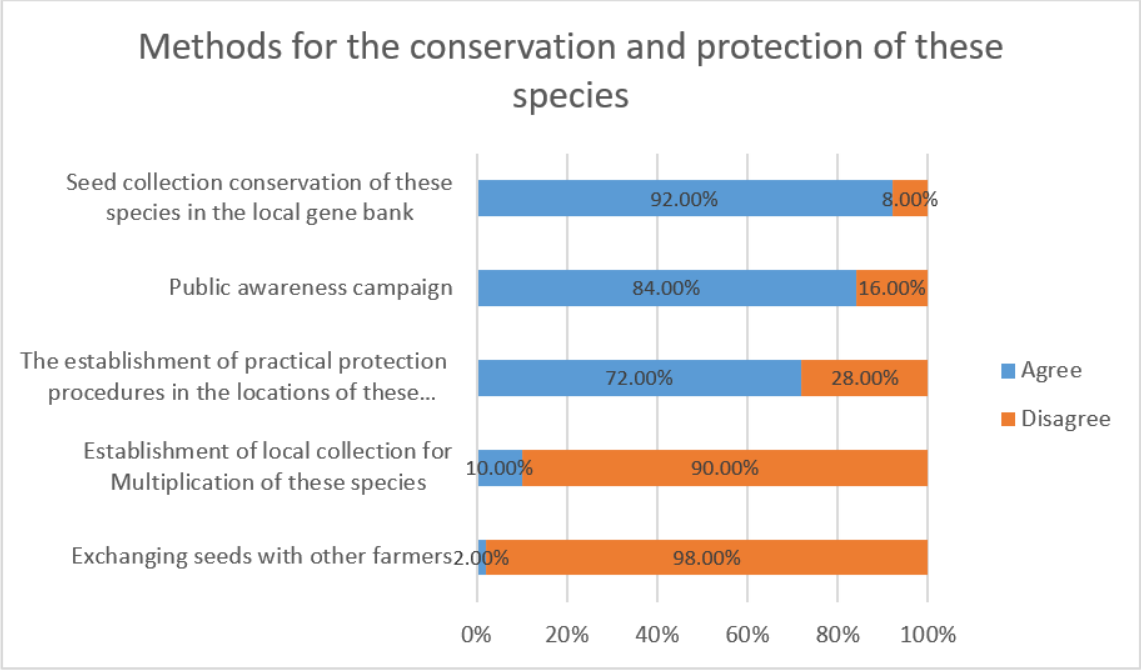

Figure 4. The proportion of respondents supporting each proposed method for the protection of medicinal species (N=50).

conservation efforts. In contrast, some of the other six species were reported as deteriorating in only one or two subdistricts, though the responses do not clarify the reasons for this. It could be due to more intense overharvesting in specific districts, but further investigation is needed to identify other potential causes.

The data might also suggest that certain species are naturally absent from areas where they were not mentioned, indicating a restricted distribution that also calls for conservation attention. Alternatively, uneven distribution could reflect increasingly unsuitable conditions in particular subdistricts for specific species. For instance, watercress, which requires freshwater streams, may be especially vulnerable to droughts in certain districts. A more comprehensive understanding of these patterns will help target conservation actions more effectively, ensuring that future plans are tailored to meet the specific needs of each subdistrict.

The questionnaire also examined the conservation actions that respondents considered most appropriate. It was encouraging to find that nearly all respondents agreed on the importance of seed collection for the establishment of genebanks. This indicates broad support for the seed collection activities conducted as part of this study. However, there was less enthusiasm for practical *in situ* conservation methods, which may be due to perceptions that such actions are difficult or unfeasible in a conflict zone. For proper tracking and future use, each seed sample was labelled with essential 'passport' information, including the species name, original collection location, and collection date, which includes the date the seeds were placed in storage. The seeds were then stored in paper bags, which were placed within airtight plastic containers along with dry silica gel to maintain optimal humidity levels at ambient room temperature (approximately 20–25°C). Seed samples have been temporarily stored in a designated room at the field staff's home

The research team acknowledges that ambient temperature storage is not a suitable long-term conservation method, as it may lead to degradation over time. This decision was made due to limited access to cooling systems in Northwestern Syria, as well as immediate access requirements and preliminary storage needs. However, the team fully recognizes the limitations of this approach for long-term preservation. To address this, the team is exploring alternative conservation strategies. These may include transferring the samples to optimal cold storage conditions and collaborating with official authorities, such as agricultural research centres or the Faculty of Agriculture in Idleb Governorate, to ensure the long-term viability and accessibility of these samples for future research

The findings of this study align with previous research on the deterioration of plant genetic resources and the conservation of medicinal plants. They reveal a consistent decline in biodiversity, particularly in regions like Syria, where climate change, habitat destruction, and socio-political instability – especially the ongoing conflict – accelerate the loss of valuable plant genetic resources. These results echo earlier studies documenting similar patterns of biodiversity loss due to environmental and socio-political challenges. The study underscores the urgent need to preserve medicinal plants, which are vital for both medicinal and cultural purposes. Environmental degradation, climate change, and conflict have significantly accelerated the decline of

plant biodiversity, emphasizing the necessity for targeted conservation efforts.

In Northern Syria, the ongoing crisis, coupled with the lack of effective conservation measures, has placed medicinal plants at significant risk of extinction. This poses a threat not only to the region but also to the global community. The conservation of these plants is essential for healthcare, biodiversity, sustainable ecosystems, and cultural heritage. Furthermore, the study highlights the critical need for immediate action to safeguard these plants, pointing to the absence of engagement from conservation organizations, which worsens their vulnerability.

Knowledge of the drivers behind the deterioration of these species is key to the development of appropriate conservation actions. The results of the questionnaire provide some useful pointers with the cause most frequently named by the respondents being 'massive unmanaged gathering' followed by 'frequent droughts'. Whilst recognizing the difficulty of implementing any management of the level of collecting whilst in a war situation, the results of the questionnaire nevertheless highlight the need to develop and implement a policy that will control and sustainably manage these key genetic resources. Although the Syrian crisis was only listed by 5% of the respondents as a cause of the deterioration, when participants were directly asked whether the Syrian crisis contributed to the deterioration of medicinal plants, with options for 'Yes' or 'No', 60% of the respondents considered it to have been a contributory factor which is closely associated with excessive use, characterized by unmanaged gathering of plant material. It also seems likely that the crisis impacts medicinal plant populations indirectly: for example, the supply of pharmaceutical drugs has been compromised by the crisis and has probably led to increased unmanaged gathering of medicinal plants by vulnerable people in search of cost-effective treatments.

While the crisis is a significant factor, the decline of plant genetic resources in Syria began before the war that started in 2011 (FAO, 1996; Syrian Government, UNDP, GEF, 2009). Respondents noted that, although overuse is the primary cause of this decline, other contributing factors include frequent droughts, climate change, desertification and the expansion of urban development into forested areas. Moreover, neglect by local authorities, a lack of awareness about the importance and value of these species, and insufficient law enforcement further exacerbate the issue. Additionally, there is a noticeable disinterest among the younger generation in using plants for medicinal purposes, potentially due to the unavailability of certain species in their natural habitats.

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Our findings point to a substantial gap in policy regarding Syria's indigenous genetic resources. While we have taken concrete steps to preserve what remains, there is an urgent need to develop a comprehensive national strategy to protect these species. Such a strategy should include both *in situ* and *ex situ* seed conservation. Also, awareness campaigns are essential to emphasize the importance of these plants, and the implementation of protective measures in the areas where they are found. These measures should be designed to specifically address the factors driving the decline of these species, with protocols tailored to reflect regional differences and local conditions. Only by adopting a comprehensive approach can we ensure the long-term survival and genetic diversity of these invaluable medicinal plants.

Supplemental data

Questionnaire including responses received in the survey

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Author contributions

Munzer Aldarvish coordinated the research and contributed to the research design and manuscript; Anas Al Kaddour, Akram Bourgol and Yasser Ramazan contributed to the research design and manuscript and undertook the data analyses; Yousef Hallak contributed to the research design and carried out the field data collection; Stephen Caversand Joan Cottrellprovided academic guidance and support throughout the research process and contributed to the manuscript.

Conflict of interest statement

The authors of this manuscript have no conflicts of interest to declare. All co-authors have seen and agreed with the manuscript's contents, and there is no financial interest in the report.

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Overview of germplasm collecting activities for plant genetic resources for food and agriculture in Sudan from 2002 to 2022

Ibrahim Mohamed El Tahir *, Ali Zakaria Babiker, Elsadig Ahmed Abdalla, Awadelkarim Alam Elhuda Ahmed, Mashaer Obaed Yousif Goda and Magdi Ahmed Mohamed Elgabri

Agricultural Plant Genetic Resources Conservation and Research Centre, Agricultural Research Corporation, Sudan

Abstract: From 2002 to 2022, the Agricultural Plant Genetic Resources Conservation and Research Centre (APGRC) in Sudan conducted 56 collecting missions for plant genetic resources for food and agriculture (PGRFA) in Sudan. These missions aimed to conserve the country's crop genetic diversity and covered different states and almost all ecological zones within the country, from the desert in the north to the high-rainfall savannah in the far south. Different farming systems were included, such as rain-fed, irrigated and flood-irrigated systems. The most covered states were West Darfur in the far west followed by South Kordofan in the western-central region. A total of 7,720 PGRFA accessions were collected encompassing diverse crops and plant species within different plant groups. Cultivated varieties made up 90% of the whole collection, while crop wild relatives accounted for 8%, and range plants represented the remaining 2%. Cereals were the most collected group (48%), followed by vegetables (17%). The least represented groups were range plants, medicinal plants and fibre crops. Sorghum was the most represented crop in the collection with 2,481 accessions, followed by pearl millet with 1,022 accessions. Hundreds of accessions of cowpea, okra, sesame and other crops were also collected. A total of 181 accessions of natural range plants were collected from selected states. The materials collected during these germplasm collecting missions will be conserved at the APGRC genebank, characterized and evaluated for different traits. Further germplasm collection activities may be carried out in the future to address any identified gaps.

Keywords: PGRFA, collecting missions, conservation, cultivated varieties, crop wild relatives, range plants, South Kordofan

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Introduction

Sudan is the third largest country in Africa, after Algeria and the Democratic Republic of Congo, with an area of 1.88 million km^2 (UNEP and HCENR, 2020). It is located between latitudes 10° and 22° North, and longitudes 22° and 38° East. Sudan is ecologically divided into five vegetation zones according to rainfall patterns from north to south (Figure 1). These are: 1) Desert (0–75mm), 2) Semi-desert (75–300mm), 3) Low-rainfall savannah on clay or sand (300–800mm), 4) High-rainfall savannah (800–1500mm) and 5) Mountain vegetation (300–1000mm) (HCENR, 2015).

According to the Ministry of Agriculture and Forestry (2015), Sudan's cultivable arable land is estimated at 86 million hectares. However, less than 20% of the potential area is utilized in three major farming subsectors (Ministry of Agriculture and Forestry, 2015):

- The irrigated system subsector, estimated at 5 million hectares, where 100% of wheat and 25% of sorghum are produced, in addition to others including vegetables and fruit trees, oil crops such as groundnut, and cotton as a cash crop;
- The semi-mechanized rain-fed subsector, estimated at 6 million hectares, where the two main

*Corresponding author: Ibrahim Mohamed El Tahir (eltahir81@yahoo.com)

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crops are sorghum and sesame, in addition to sunflower;
- The agro-pastoral traditional rain-fed subsector, of about nine million hectares, where main crops produced are sorghum, pearl millet, sesame and groundnut, as well as seed watermelon and roselle.

Sudan is endowed with different ecosystems making it rich in biodiversity, including plant genetic resources for food and agriculture (PGRFA). Sudan is part of the East African Primary Region of crop genetic diversity, with high diversity for coffee, cotton, cowpeas, melons, millets, olives, peas, sesame and sorghum (Khoury *et al*, 2016). Wild relatives of crops such as sorghum, pearl millet, okra, watermelon and melon are known in the country (Mahmoud *et al*, 1995). Old introduced cultivars for crops such as maize, faba bean and tomato are still existing and utilized by some farmers. In addition, rangelands encompass different ecological zones extending from desert and semi-desert in the north to low and high rainfall savannah to the southern border and include a wealth of natural range plant species (HCENR, 2013).

Factors threatening the genetic diversity of PGRFA in Sudan include several natural and man-made factors. The most important ones are drought spells and seasonal rain fluctuations, expansion in modern agriculture and use of advanced improved cultivars, expansion in new constructions and activities other than crop cultivation, and disturbance of normal traditional systems of life, including agricultural activities due to civil strives and wars in several regions within the country (HCENR, 2015).

In order to safeguard these important genetic resources, the Agricultural Research Corporation (ARC) of Sudan established the Plant Genetic Resources Programme (PGR Programme). Historically, the PGR Programme dates back to the early 1980s, when activities for collecting and conserving local genetic resources of horticultural crops were conducted by the Horticultural Research Section of the ARC in collaboration with the International Board for Plant Genetic Resources (Hassan *et al*, 1983). In 1995, the programme was mandated for collecting, conserving and enhancing the use of the genetic resources for different agricultural crops under what was by then called the Plant Genetic Resources Unit. Following expansion in the total germplasm holdings and the human and physical capacities of the programme, this unit was upgraded in 2014 into a centre now known as the Agricultural Plant Genetic Resources Conservation and Research Centre (APGRC), which is currently managing the PGR programme. The APGRC headquarters are located in the city of Wad Medani in central Sudan.

During the 1990s and 2000s, the coverage of PGRFA acquired by the PGR Programme expanded to different groups such as cereals, oil crops, grain legumes, in addition to horticultural crops including vegetables, fruit-producing plants and medicinal and aromatic plants. Significant progress in the efforts to collect and acquire PGRFA from different sources occurred from 2003, thanks to the availability of necessary financial resources to conduct germplasm collecting missions from different regions, as well as the improvement in the physical and human capacities of the PGR unit. Funding for the PGR Unit came from the Eastern Africa Plant Genetic Resources Network (EAPGREN) through a project funded by the Swedish International Development Cooperation Agency (Sida), which extended up to 2010, and other funding sources such as the Global Crop Diversity Trust (Crop Trust). Moreover, the budgetary payments to the PGR programme were significantly increased by the national government on an annual basis since 2014.

This paper is intended to provide a general overview of the collecting activities conducted under APGRC, and summarize the results obtained over 20 years from 2002 to 2022 for collecting indigenous PGRFA within Sudan for conservation in the APGRC genebank facilities. Those genebank facilities consist of seedbanks, and field genebanks for vegetatively propagated crops. Information on germplasm collecting activities has been documented within the APGRC genebank documentation system, as well as in the global PGR data platform Genesys (http://www.genesys-pgr.org/wiews/SDN002). This review provides information on the methodologies used to collect PGRFA, the locations visited, the germplasm materials collected as well as the phenotypic variations observed on the collected materials. The results are thoroughly discussed to explain the extent and importance of the geographical coverage and the PGRFA materials collected. The conclusion highlights the major achievements from the germplasm collecting activities and identifies major gaps to be addressed in the future.

Methodology

The germplasm collecting activities followed a structured process, starting with planning and ending with germplasm sampling and data recording on the collected PGRFA from the targeted geographical locations before their conservation at the APGRC genebanks.

Planning

Planning for germplasm collection was conducted through consultative processes involving all APGRC scientific staff during regular programme planning activities within the ARC national research programme planning process. Geographical locations and taxa for collecting were identified and prioritized based on the following:

A. Geographical prioritization

Ecological zones and geographical areas were selected and prioritized based on the following criteria:

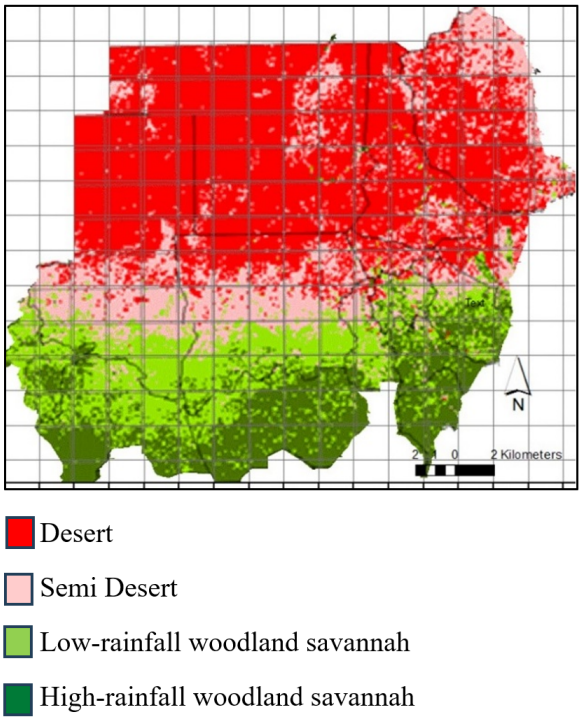

Figure 1. Map of Sudan vegetation zones (Eltoum *et al*, 2023)

1. Geographical areas known for their richness in PGRFA diversity across different ecological zones in the country. This was the case for Kordofan and Darfur regions in western Sudan, Blue Nile State in central to eastern Sudan, River Nile and Northern States in northern Sudan.
2. Major geographical gaps that were identified after 2014 in the Darfur region in far western Sudan.

B. Taxonomical prioritization

Different crops and plant taxa for collection were identified and prioritized according to the following criteria:

1. Specific taxonomical gaps within the existing germplasm collections for specific crops already held by APGRC, as identified in consultation with different crop specialists, such as for crops like sorghum and pearl millet.
2. Overall taxonomical gaps in terms of crops and plant groups almost or fully absent within the APGRC collection, as was the case for taxa and plant groups such as banana, date palm, crop wild relatives and natural range plants.

Detailed collection work plans were developed annually by the APGRC researchers in consultation with the APGRC technical staff and/or directors of the ARC stations in the targeted regions, as well as with technicians and local communities there.

The whole processes of planning at different stages were coordinated, guided, supervised and approved by the APGRC director, who afterwards closely followed the implementation processes.

Targeted PGRFA

Regular germplasm collecting missions were organized annually across the different cultivation seasons starting from season 2002–2003 to season 2013–2014. They targeted different PGRFA, including cereals such as sorghum and pearl millet; oil crops such as sesame and groundnut; legumes such as cowpea and faba bean; vegetables and cucurbits such as okra, tomato, melons and watermelon; medicinal and aromatic plants such as roselle and fenugreek, and fruit producing plants such as banana and date palm. Starting from 2014, the focus was on filling geographical and taxonomical gaps, of which the latter included date palm, natural range plants and crop wild relatives.

Targeted locations

Germplasm collecting activities targeted various farming systems in the different ecological zones during the appropriate seasons, including rain-fed, irrigated and river-flooded systems. The administrative states of the country were the broad targeted geographical locations for the different collecting missions. The ecological zones covered across the different states included (Supplemental Table 1):

- The desert and semi-desert zones in Northern, River Nile, Khartoum, Kassala and Red Sea States;
- The low-rainfall savannah on clay and sand in the States of Gezira, Sennar, Blue Nile, Gedarif, and in the states within Kordofan and Darfur regions;
- High-rainfall savannah in southern parts of the Blue Nile State, and Kordofan and Darfur regions.

The major geographical gaps targeted since 2014 were the different states within the Darfur region of far western Sudan, which was difficult to cover earlier because of the civil war since 2003.

Means of transportation to these locations was usually a 4 WD vehicle facilitating reaching to different collection points that were usually away from asphalt roads. However, they were accessible under the guidance of the representatives of the local authorities and communities within the collecting teams.

Collecting approaches

The collecting missions were implemented following either multi-species or single-species collecting approaches. The cultivation season in Sudan usually starts in summer from May to October, and extends to winter from November to April. Therefore, the germplasm collecting activities from *in situ* habitats were usually carried out during the harvesting times, which were from October to December or beyond for summer-adapted crops such as sorghum, sesame, cowpea and okra; and from February to April for winter-adapted crops such as wheat, faba bean and tomato.

Multi-species collecting missions

A total of 39 multi-species collecting missions were organized across 17 years during the period extending

Table 1. Approaches and total numbers of collecting missions conducted from 2002 to 2022. More details are available in Supplemental Tables 1 and 2.

Approach	Targeted plants	Total
Multi-species	Different crop groups	27
	Vegetables	1
	Crop wild relatives	8
	Range plants	3
	Sub-total	**39**
Single-species	Banana	11
	Date palm	4
	Sorghum	1
	Watermelon	1
	Sub-total	**17**
Grand Total		**56**

between the cultivation seasons of 2002–2003 and 2021–2022, with the exception of three seasons (2009–2010, 2011–2012 and 2012–2013), with at least one mission per year (Table 1 and Supplemental Table 1). Those missions covered different areas of the country and targeted different crop and plant species, including in some years vegetables, crop wild relatives or natural range plants (Supplemental Tables 1 and 2).

Single-species collecting missions

Seventeen single species collecting missions were organized during the specified period. They targeted sorghum, watermelon, banana and date palm from different states (Supplemental Table 1, Table 1).

Collecting teams

Each team carrying out a collecting mission was usually headed by an APGRC researcher and might include a collaborating or assisting scientist and/or a technician from APGRC or ARC (Supplemental Table 1). Missions conducted since 2014 in the states of the Darfur region were led by directors of the agricultural research stations in such states. Each team also included a representative from the State Ministry of Agriculture, and a representative from the local community in the targeted area.

Germplasm sampling

Germplasm samples were collected from farmers' fields, wild habitats or rangelands. Each population at each specific site was sampled separately with a basic approach targeting around 50 plants per population. However, for populations with fewer than 50 plants, only the available plants were sampled.

Materials collected included seeds, panicles, fully ripe or dry fruits in the form of soft berries, pods or capsules, corms for banana, and off-shoots for date palm (Figure 2). Seeds were then extracted and processed for long-term conservation in the seedbank, while banana corms and date palm off-shoots were prepared and planted in the field genebanks.

Data recorded

A germplasm collection form was used to record passport data on each sample collected (Supplemental Material 1). The form was derived from the FAO-IPGRI Multi-Crop Passport Descriptors (FAO and IPGRI, 2001). It consisted of major information such as taxonomic data, georeferenced data on the site of collection, date of germplasm collection, and local names. However, a specific germplasm collection form including more descriptors was used for collecting crop wild relatives during 2016 and 2017 (Eastwood *et al*, 2022). GPS was used to determine the coordinates and altitudes of the collection sites.

Results

Results obtained through the collecting activities across the cultivation seasons from 2002–2003 to 2021–2022 are summarized in terms of geographical locations covered, germplasm materials collected and phenotypic variations observed on the collected samples.

Missions conducted and sites covered

A total of 56 germplasm collecting missions were conducted, including 39 for multiple species and 17 for single species (Table 1). Among the single-species missions, 11 and 4 targeted banana and date palm genetic resources respectively, while sorghum and watermelon were targeted each by only one mission in Delta Elgash in Kassala State, and North and West Kordofan States, respectively.

Germplasm samples were collected from 1,155 sites within the 18 states of Sudan (Supplemental Table 2). They were located within latitudes ranging between 09.52° N in South Kordofan State and 20.93° N in Northern State; and longitudes ranged between 21.85° E in West Darfur State and 38.43° E in Red Sea State (Figure 3). In terms of collection sites, the most covered states were West Darfur and South Kordofan, where germplasm entries were sampled from 142 and 135 sites, respectively. The least covered states were Sennar and Khartoum, where germplasm entries were sampled from five and nine sites, respectively.

The number of states contributing to the total accessions of each crop varied considerably between the different crops, reflecting the diverse growth habitats and requirements. While okra (*Abelmoschus* spp.) accessions were sampled from 17 states, and sorghum (*Sorghum* spp.) and roselle (*Hibiscus sabdariffa*) were sampled from 16 states each, genetic resources of 20 crops were sampled from only five states or less, including the date palm accessions, which were sampled from only two states (Supplemental Figure 1).

Germplasm materials collected

A total of 7,720 accessions were collected from more than 40 cultivated crops and other PGRFA species (Supplemental Table 3). They were composed of different crop groups including cereals such as sorghum

Figure 2. Banana and date palm materials collected

(*Sorghum* spp.), pearl millet (*Pennisetum* spp.) and maize (*Zea mays*); grain legumes such as cowpea (*Vigna* spp.), faba bean (*Vicia faba*) and chickpea (*Cicer arietinum*); oil crops such as sesame (*Sesamum* spp.) and groundnut (*Arachis hypogea*); vegetables such as okra (*Abelmoschus* spp.), tomato (*Solanum lycopersicum*), and pumpkins and squashes (*Cucurbita* spp.); medicinal and aromatic plants such as roselle (*Hibiscus* spp.) and fenugreek (*Trigonella foenum-graecum*); fruit-producing plants such as banana (*Musa* spp.) and date palm (*Phoenix dactylifera*), in addition to natural range plants. Cereal genetic resources were the most represented in the collected materials, accounting for 49%, followed by vegetables at 17%, while medicinal and aromatic plants, range plants and fibre crops were the least represented, comprising 5%, 2% and less than 1%, respectively (Figure 4).

The total number of collected accessions varied greatly between cultivated crops ranging from only one to a few thousands. Out of 47 cultivated crops covered, 12 crops were represented by more than 100 accessions of each, collected from different numbers of states that varied between different crops (Table 2), adding up to 6,656 accessions and representing 86% of the total collection. The rest (1,064 accessions) were from other cultivated crops, wild plants or natural range plants, including 38 genera of other cultivated crops and wild plants, in addition to 39 genera of range plants.

The most collected crops were sorghum and pearl millet, of which 2,481 accessions and 1,022 accessions were collected, respectively (Table 2). Hundreds of accessions ranging from 179 to 449, were collected from date palm, melons, maize, watermelon, roselle, groundnut, banana, okra, sesame and cowpea.

All 18 states of Sudan were represented in this top collection, with the total number of accessions varying from 14 in Khartoum and Sennar States to 1,550 from South Kordofan State (Table 2).

Almost 90% of the accessions collected were from cultivated varieties, while only 8% were wild, including crop wild relatives and other wild plants apart from range plants. The rest of the accessions were from natural range plants, representing 2% of the total materials collected.

Taxa of crop wild relatives fully identified ranged between five for sorghum (*Sorghum virgatum, S. purpureosericeum, S. halepense, S. bicolor - verticilliflorum* and *S. aethiopicum*), three for eggplant (*Solanum incanum, S. dubium* and *S. cerasiforme*), and two for pearl millet (*Pennisetum stenostachyum* and *P. glaucum monodii*) and melons (*Cucumis melo agrestis* and *C. metulikerus*). Others including watermelon, sesame, cowpea, and rice had only one fully identified wild species, which were *Citrullus colocynthis, Sesamum alatum, Vigna vaxillata* and *Oryza barthii*, respectively (Supplemental Figure 2).

The total accessions collected of natural range plants were 181. They included 151 accessions, fully or partially identified taxonomically, belonging to 50 species across 37 genera. However, 30 accessions were taxonomically unidentified and documented only using their local names (Supplemental Figure 3).

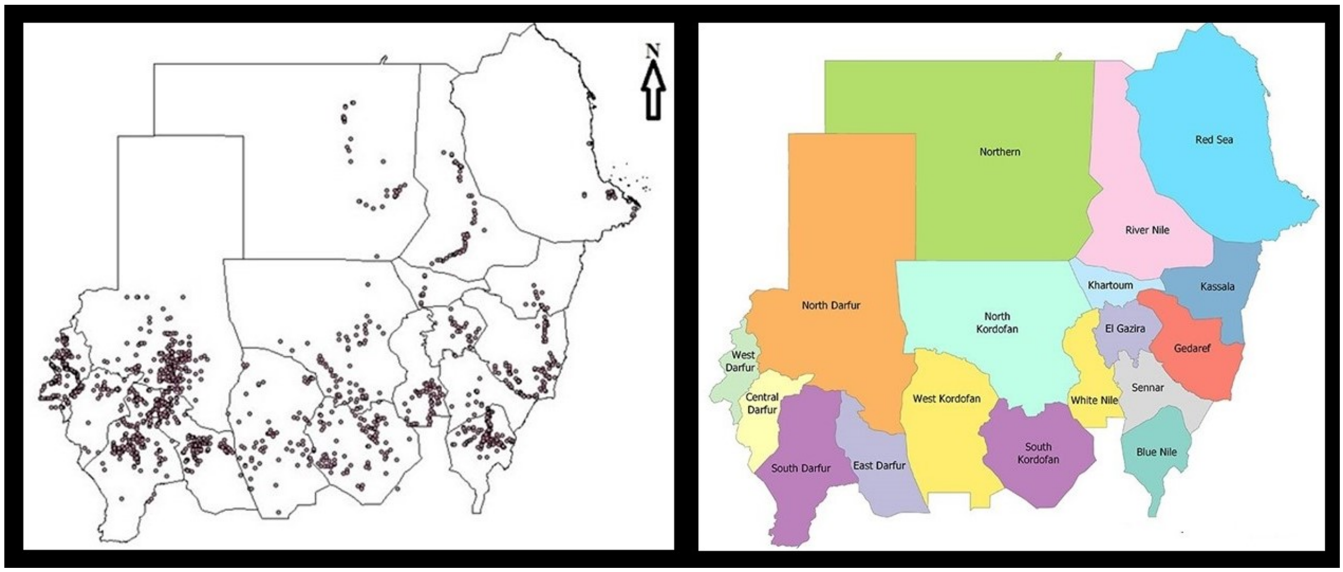

Figure 3. Sites where most germplasm accessions were collected from 2002–2022 (map A) across different states of Sudan, with state names shown on map B.

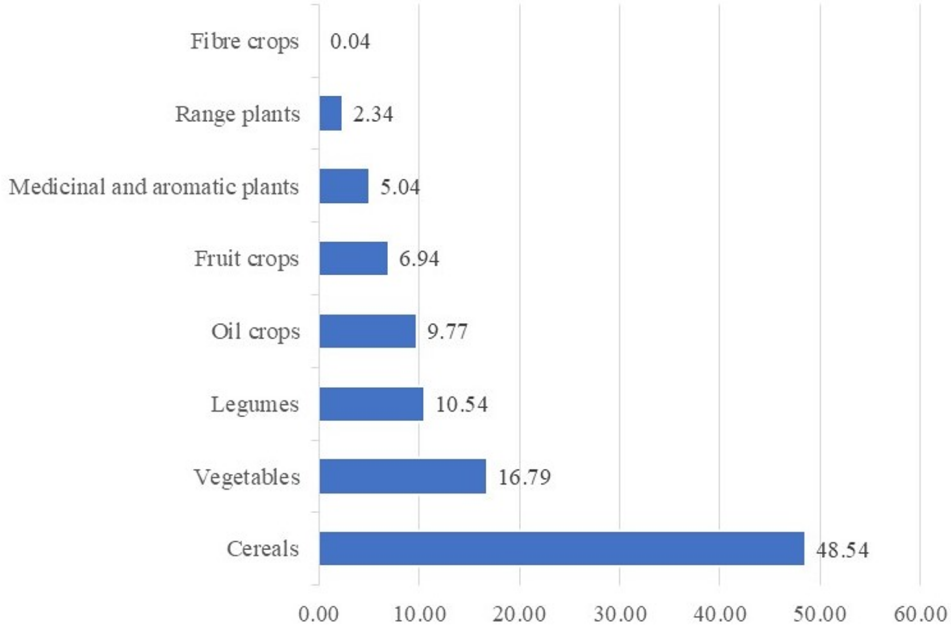

Figure 4. Distribution of percentages of total accessions collected from different plant groups

Table 2. Distribution of number of accessions of the top 12 crops collected among different states in Sudan between 2002 and 2022.

State	Sorghum	Pearl millet	Cowpea	Okra	Sesame	Banana	Groundnut	Roselle	Watermelon	Maize	Melon	Date palm	Total
South Kordofan	706	93	158	124	122	0	69	62	9	132	75	0	**1,550**
South Darfur	237	168	58	40	38	22	46	23	14	11	21	0	**678**
West Darfur	147	147	42	52	45	0	69	94	44	1	25	0	**666**
Blue Nile	172	94	57	33	68	84	27	18	0	36	10	0	**599**
North Kordofan	134	167	31	26	27	0	4	40	19	10	13	0	**471**
Kassala	273	3	2	9	2	151	2	2	10	2	12	0	**468**
East Darfur	135	49	7	16	13	0	79	54	11	0	6	0	**370**
North Darfur	144	141	11	7	21	0	13	17	7	0	0	0	**361**
Gedarif	142	35	52	13	51	0	21	6	4	9	16	0	**349**
Northern	9	0	6	16	0	21	0	2	5	11	4	139	**213**
Central Darfur	69	53	7	12	6	0	15	14	2	4	2	0	**184**
White Nile	169	3	4	1	1	0	1	2	0	0	0	0	**181**
Red Sea	89	48	0	26	0	0	0	4	0	1	13	0	**181**
West Kordofan	18	19	5	5	2	0	2	1	120	0	7	0	**179**
River Nile	28	0	7	5	0	12	0	4	3	3	0	40	**102**
Gezira	9	2	2	3	2	49	1	0	4	1	3	0	**76**
Sennar	0	0	0	0	0	14	0	0	0	0	0	0	**14**
Khartoum	0	0	0	6	0	4	0	1	0	0	3	0	**14**
Total accessions	2,481	1,022	449	394	398	357	349	344	252	221	210	179	**6,656**
Total states	16	14	15	17	13	8	13	16	13	12	14	2	

As a result of these collecting efforts and other germplasm acquisition activities, the total PGRFA accessions conserved by APGRC reached more than 16,000 accessions by 2022 compared to around 6,000 accessions in 2002, as shown in the APGRC Genebank Documentation System, with a total increase of around 10,000 accessions (Figure 5). The collecting missions reported here contributed significantly to this increase with 7,720 accessions, while the rest came from other germplasm acquisition activities during the same period, including repatriation of Sudanese germplasm from outside the country, and donations of germplasm samples by others such as crop breeders.

Phenotypic variations observed

Remarkable phenotypic variations on some morphological traits were visually observed on the plant organs collected such as the panicles, fruits and seeds. Such observations will be further studied and documented through detailed characterization using standard descriptor lists. In cereals, variations in shapes, sizes and colours were observable on panicles and seeds of sorghum, pearl millet, maize and rice (Figure 6). Seed colours of sesame and groundnut, both oil crops, were varied among the accessions collected from different sites (Figure 7). Remarkable variations were also observed in colours and sizes of seeds of some leguminous crops such as cowpea, faba bean, hyacinth bean and Bambara groundnut (Figure 8).

Variations were also observed on a number of horticultural crops, including Malvaceous and Solanaceous vegetables and medicinal plants (Figure 9). Capsule shapes were variable among the okra accessions, as well as the colours of dry enlarged calyces of roselle accessions. Fruit sizes and shapes also varied among tomato, hot pepper and eggplant accessions. Prominent variations were also observed in shapes, sizes and colours of fruits and seeds of different cucurbits such as watermelons, melons, pumpkins and squashes, and bottle gourds (Figure 10).

Discussion

The total number of PGRFA accessions conserved by APGRC before 2002 was approximately 6,000. A significant increase in the APGRC holdings occurred during the following 20 years resulting in more than 16,000 accessions by 2022. This increase of around 10,000 accessions, was primarily due to the intensive germplasm collecting activities undertaken during the cultivation seasons from 2002 to 2022, which accounted for 77% of the germplasm acquired, in addition to repatriation of Sudanese germplasm from outside the country, and donations of samples by others. On the other hand, direct collection by APGRC from *in situ* habitats contributed by only 21% before 2002, during the 20 years since the start of formal germplasm collecting activities in 1982. In fact, the horticultural germplasm materials collected in 1982, 1983 and 1985, as reported by Hassan *et al* (1983), Hassan *et al* (1984) and Geneif *et al* (1985), were the first accessions deposited in the genebank unit that later evolved to become APGRC.

The increased role of APGRC as a germplasm collecting institute after 2002 was mainly due to the availability of necessary resources from different streams, including funding for germplasm collecting. For example, the period between 2002 and 2010 witnessed a substantial increase in the resources available for APGRC from the capacity-building project for PGR under the EAPGREN network, of which Sudan was a founding member (Marandu and Kamau, 2008). This project, financed by the Swedish International Development Cooperation Agency, aimed to implement national and regional activities, including germplasm collecting activities. Also, APGRC obtained funds from a project financed through the Crop Trust from 2015 to 2017 for collecting crop wild relatives, which contributed significantly to increasing the APGRC holdings of crop wild relatives (Eastwood *et al*, 2022). Additional financial support was obtained from the Crop Trust through the CGIAR Genebank Management Platform and the International Center for Agricultural Research in the Dry Areas (ICARDA) in 2021 for collecting PGRFA to fill gaps in the collections held by APGRC and other international research centres.

The number of sites sampled was considerably large, which was made possible by reliable transportation means that enabled access to various locations, even those far from asphalt roads. Membership in the collecting teams of representatives from local authorities and communities was invaluable in guiding those teams through local internal roads connecting villages and leading to wild habitats.

Almost half (48%) of the accessions collected from 2002 to 2022 were cereal crops, of which sorghum was represented by 66%, and pearl millet by 27%. This highlights the importance of cereals, especially sorghum and pearl millet, in the country in terms of the extent and distribution of cultivated areas, as well as the diversity they encompass, including farmers' cultivars and wild relatives. Both crops are major staples in Sudan, and are annually cultivated in areas of more than 10 million hectares for sorghum, and 4 million hectares for pearl millet (Ministry of Agriculture and Forestry, 2015). Moreover, Sudan is part of the primary diversity regions for these crops in East and West Africa, as indicated by Khoury *et al* (2016). It is located within sub-Saharan and Northeast Africa, which is the primary centre of origin and diversity of sorghum as mentioned by Barmel *et al* (2022b). Pearl millet was domesticated in West Africa and then diffused into eastern Africa, southern Africa and South Asia (Barmel *et al*, 2022a). Therefore, eastern Africa, including Sudan, contains remarkable variability in pearl millet as was observed in the number of pearl millet accessions collected by APGRC and the variations observed. This was proved by Bashir *et al* (2014), who identified clear genetic

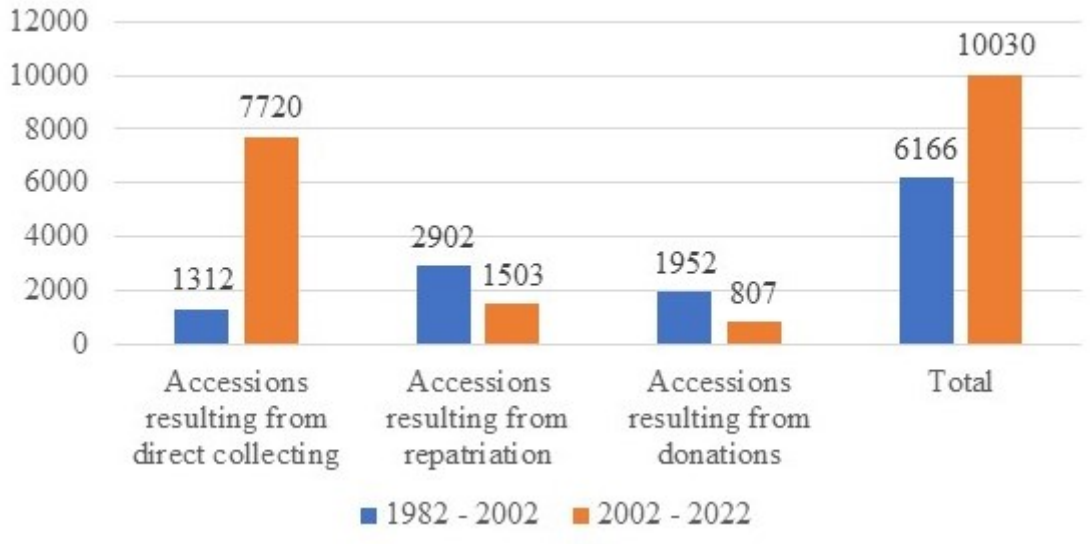

Figure 5. Comparisons between total accessions acquired by the Agricultural Plant Genetic Resources Conservation and Research Centre (APGRC) from different sources during the periods before and after 2002

Figure 6. Variations among panicles and seeds of different cereal crops accessions (sorghum, pearl millet, maize and rice) collected from different Sudan states during different seasons

diversity within a sample of 214 accessions from Sudan, using marker-based analysis.

Vegetables, including Malvaceous, Solanaceous and Cucurbit crops were the second largest group among the materials collected, representing 17%. Various vegetables are consumed in Sudan; among them are wild species, indigenous vegetables and introduced types, as mentioned by Ahmed and Mohamed (1995). Among the collected vegetables, true indigenous forms of okra, melons and watermelons were present showing remarkable morphological variation. One piece of evidence of the geographical origin of okra is partially based on the presence of a putative ancestor (*A. ficulneus*) in East Africa (Kumar *et al*, 2011). Schippers (2002) reported that *A. ficulneus* was found in Sudan and other regions of the Sahel and East Africa, but more so in South and South East Asia. Okra is one of the most important traditional vegetables in Sudan that is used almost all over the country in a number of ways of cooking either after being dehydrated or as fresh pods (Mohamed, 1991). This explains the widest geographical range from which okra genetic resources were collected making it the leading crop in terms of the number of states (17) from which samples were obtained. Variations observed among okra fruits collected were further shown on vegetative, inflorescence and fruit traits through morphological characterization results as recently reported by El-Tahir (2023).

The collections made over 20 years contained a considerable number of melon accessions totalling 210, including cultivated and wild melons with observed

Figure 7. Variations among seeds of different oil crops accessions, including sesame and groundnut, collected during different seasons from various Sudan states

variations among them. Mohamed and Yousif (2004) described Sudan as unique, in terms of the presence of different melon subspecies, containing both wild and cultivated genotypes. Cultivated melons in Sudan are composed of sweet melon, known locally as Shammam; snake melon, known locally as Ajjour; a local vegetable melon named 'Tibish'; and a local type of melon of which seeds are eaten, known locally as Seinat. Wild melon plants (*C. melo agrestis*) are also found in Sudan and are known as Humaid. Among them are types of melon that are truly indigenous to the country, either of typical wild type such as *C.melo agrestis*, or grown only in Sudan such as Tibish (Mohamed and Pitrat, 1999). In contrast, cultivars of sweet melons and snake melons are introductions from outside the country. According to Pitrat (2013) the Tibish melon is considered a primitive cultivated melon that evolved and domesticated independently in a domestication event separate from other cultivated melons in Africa and belonging to the subspecies agrestis. The latter subspecies agrestis was considerably represented in its wild form in the collections made from 2002 to 2022 by 153 accessions, providing supportive proof of the probability that Tibish melon might have been domesticated in Sudan through evolution from such wild forms. This collection is uniquely important since the wild and cultivated agrestis melons Tibish and Humaid have been proved to be sources of resistance to a number of viral and fungal diseases, as well as insect pests, as reviewed by Mohamed and Yousif (2004).

The collecting efforts resulted in 252 accessions of watermelon, of which more than 50% were from Kordofan region. One of the reasons for the importance of this collection is the recently reported information by Renner *et al* (2021), indicating that the closest relative to the domesticated watermelons was the Kordofan melon (*Citrullus lanatus* subsp. *cordophanus*) from Sudan. An earlier study by Goda (2007) on morpho-

Figure 8. Variations among seeds of different accessions of leguminous crops, including cowpea, faba bean, hyacinth bean and Bambara groundnut, collected during different seasons from different Sudan states.

Figure 9. Variations among accessions of Malvaceous crops, including capsules of okra and dry calyces of roselle, as well as among fruits of the Solanaceous crops tomato, hot pepper and wild relatives of eggplant, collected from some Sudan states during different seasons

Figure 10. Variations among fruits and seeds of some accessions of cucurbit crops, including watermelon, melon, pumpkin and bottle gourd, collected during different seasons from different Sudan states

logical characterization of 28 *Citrullus* accessions from Sudan, revealed remarkable variations shown by clustering analysis distinguishing between four main distinct groups. The first group was characterized by 88% similarity within the group members and consisted of cultivated accessions collected mainly from Kordofan and Darfur regions in the west. This group was further divided into three subgroups indicating the variation of the genetic resources of this group within a region, from where watermelon is believed to have originated (Goda, 2007).

Among the Solanaceous vegetables collected were tomatoes, hot peppers and eggplants. All of them originated in regions far away from Sudan but have been introduced into the country some time ago. An earlier study on tomato accessions collected from the western and northern regions of Sudan showed distinctive features of each compared to the other (El-Tahir, 1993). Tomatoes from western Sudan were mostly small or very small in size and were mostly used for making dried tomato paste from fruit slices. On the other hand, tomatoes from the Northern region of Sudan were medium or relatively big and mainly used as salad tomatoes. Such wide variations in tomato fruits were proved to be still there as shown within the collections obtained from 11 states, including from western and northern Sudan. Capsicum species originated in South America from where they dispersed into different regions of the world forming secondary centres of diversity, among which eastern and southern Africa, where Sudan lies. West and Central Africa represent a significant secondary region of diversity for *C. chinense*, while East Africa is likely to be an important secondary region of diversity for *C. frutescens* (Barchenger and Khoury, 2022); both hot pepper species were present in Sudan as suggested by El-Tahir (2001), and, therefore, are likely to have been collected during the last 20 years. The popularity of this crop and its production in smallholdings, in diverse environmental conditions of different regions substantially contributed to the observable vast variability of this crop in Sudan (Geneif, 1984). This variability was further confirmed by El-Tahir (1994), who indicated the availability of high variation between and within 116 accessions from Sudan in plant and fruit characters. Accordingly, it was recommended to have further collections of hot peppers to cover new areas. The collections made after 2002 resulted in dozens of accessions from nine states in response to this earlier recommendation.

Among the food grain legumes, the total collected accessions of cowpea ranked third after sorghum and pearl millet with 426 accessions, originating from different states in the west and south-east with clear phenotypic variations of the collected seeds (Figure 8). In a study on cowpea accessions from Sudan, collected in the last 20 years, Sudanese germplasm showed high similarity to West African cowpeas (Ali *et al*, 2015). This was interpreted by an earlier suggestion that the cowpea crop was introduced to Sudan from West African countries to the western part of Sudan (Kordofan and Darfur), from where it spread to the rest of the country (Ali *et al*, 2015). This is further supported by

the relatively large collection obtained from Kordofan and Darfur regions in Sudan.

A considerable number of accessions of oil crops sesame and groundnut were collected especially from the States of South Kordofan, South Darfur, East Darfur and West Darfur in the west of the country, and Blue Nile and Gedarif States in the southeast and east of the country, the traditional production areas for such crops. The sesame collection was relatively abundant, ranking fourth among the top collected crops from 13 states, encompassing mostly farmers' cultivars with about 10% of wild sesame species. Such a relatively big collection of sesame from a wide geographical distribution with observable variation in seed colours is indicative of the broad diversity of sesame genetic resources in Sudan, similar to that of cowpea and okra. This could be attributed to the fact reported by Mahmoud *et al* (1995) that the selection by subsistence farmers resulted in many landraces of sesame adapted to different ecological areas, varying mainly in rainfall and soil, and to the needs of the farmers. Sesame was suggested to have been domesticated in Africa (Bedigian, 2004), as several wild relatives are still growing there. Sudan is one of the countries in sub-Saharan Africa where a variety of cultivated and wild sesame is found, as proved by the germplasm collecting activities reported here. This indicates the importance of the country as a place of diversity for this crop, and suggests the possibility that it may be one of the regions where sesame was domesticated.

As discussed by Williams (2022), the groundnut is believed to have originated in South America in the area of southern Bolivia and northwestern Argentina. It was introduced to West Africa by Portuguese traders in the 16^{th} century, and in the following centuries it became fully integrated into subsistence farming systems and various ethnic cuisines, causing the crop to diversify into dozens of distinct African landraces. West African immigrants are believed to have brought the crop to Sudan about 200 years ago, and they grew it in parts of western Sudan and along the Blue Nile (Mahmoud *et al*, 1995). This is supported by the germplasm collecting activities resulting in a relatively large collection of locally cultivated varieties of groundnut, the absolute majority of which was from Darfur and Kordofan regions in the west, and the Blue Nile State in the southeast of the country.

According to what was mentioned by McClintock and El-Tahir (2004), roselle (*Hibiscus sabdariffa*) probably originated from Africa, where it may have been domesticated in Sudan about 6,000 years ago. It is locally known as Karkade and is grown in various parts of Sudan, particularly Kordofan and Darfur, where it is one of the cash crops cultivated by traditional farmers under rain-fed conditions, for local consumption and export (Mohamed *et al*, 2012). It is therefore understandable that it ranked among the top crops, with a relatively large number of accessions collected, totalling 344. The absolute majority of these were from Darfur and Kordofan States, where the crop is traditionally grown. The variation observed during collection, especially on enlarged calyces, which are the main parts of the plant used to make cold or hot beverages, is indicative of high diversity to be detected through morpho-agronomic and genetic characterization studies.

The vegetatively propagated fruit-producing crops represented in this collection were banana and date palm. Banana is a plant that is grown in Sudan for its sweet-flavoured fruits that are used as dessert by Sudanese people. It is produced commercially in small and medium scattered orchards along the River Nile and its tributaries banks, and in large plantations at Kassala (Elsadig, 2014). The Dwarf Cavendish has been the only old cultivar grown in Sudan for about 100 years (Mahmoud *et al*, 1995). However, new banana cultivars were introduced, tested and released in the early 2000s, and might replace the traditional cultivar for their superior yield potential and quality. The collection of local banana germplasm was planned to safeguard it against any possible losses due to the use of the new improved varieties or any other factors, including the recently frequent seasonal flooding of the rivers over the banks where banana plantations are established. Therefore, the collecting activities since 2003 have reasonably covered the different areas where bananas used to be grown. The highest number of accessions (151) came from Kassala State, where banana production is believed to have started during the late 18th century and is considered historically one of the most important banana-producing centres in Sudan (Elsadig, 2014).

Date palm has been grown in Sudan for a long time in the northern region using local cultivars producing different types of dates, including dry, semi-soft and soft dates. A study to describe the phenotypic vegetative and fruit characters of a number of date palm cultivars at on-farm level in northern Sudan has revealed high variability between these cultivars, where 14 out of 16 qualitative and quantitative traits used showed a strong discriminating factor (Elsafy *et al*, 2015). Genetic variation has also been detected within and between 18 cultivar groups obtained from northern Sudan as reported by Elsafy *et al* (2016). Findings of a study reported by Ezebilo *et al* (2013) revealed that the date palm cultivars in northern Sudan are diverse, implying that date palm farms in Sudan can also serve as sites for conserving genetic resources. However, having an *ex situ* collection of such diverse date palms in Sudan has been an APGRC strategy to back up collections as a means to safeguard such materials against hazards at on farm level. Following that, the collection of date palm accessions from Northern and River Nile States began in 2015 resulting in materials from both states for conservation in a field genebank.

Rangelands in Sudan are estimated at 68.6 million hectares or 36% of the total country area (Ministry of Agriculture and Forestry, 2015). Surveys conducted

from 2000 to 2014 revealed that certain important range plant species were becoming scarce or extinct and some areas were invaded by unpalatable plant species. Thirteen valuable herbaceous species were reported as decreasing in semi-desert and low-rainfall savannah zones. Among these, were species belonging to genera such as *Andropogon, Aristida* and *Blepharis* (HCENR, 2013). The APGRC's collection of genetic resources of range plants started relatively late, in 2015. The areas surveyed for collection were limited to only three states in the semi-desert and low-rainfall savannah zones, indicating the pressing need for intensive collecting efforts in the future for range plants as an important component of the PGRFA in the country.

It is interesting to note that South Kordofan State was significantly represented in the collection of the top twelve crops, with a total of 1,550 accessions, a number much higher than that of the second-ranking state, South Darfur, which contributed 683 accessions. This can be attributed to the fact that South Kordofan was one of the states most intensively covered during germplasm collection, with 135 sites visited. Additionally, South Kordofan is known for its high diversity of cultivated crops grown in different farming systems including what is known as the 'Jubraka', the local name of kitchen home gardens, as well as in small lands held by smallholder farmers locally known as Bildat. Moreover, materials were collected from sites located at a wide range of altitudes from 117 to 976 masl. On the other end, Sennar State, which is part of the central clay plains in Sudan, was the least covered in terms of crops collected, as only banana clones were collected from the farms along the river banks of the Blue Nile. This makes Sennar State among the top geographical gaps for germplasm collection in the future. Planning for multi-species collecting from Sennar should consider areas around and within the Dinder National Park, which is one of the largest protected areas in the country covering about 890,000 hectares (UNEP and HCENR, 2020). It stretches in areas within Sennar, Gedarif and Blue Nile States, with the biggest area within Sennar State. This will require close collaboration with the park authorities for effective targeted germplasm collection within and around the park.

Around 65% of the accessions collected (4,982 accessions) were from crops and species covered by the Multilateral System (MLS) for Access and Benefit Sharing established by the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) (FAO, 2009). The total crops belonging to this system that were sampled were 22 accounting for 43% of the total crop genera covered. Such a relatively big number of accessions collected from the MLS crops and species indicates the importance of this collection from a global perspective, as Sudan is a party to the ITPGRFA and obliged to provide facilitated access to such accessions based on the MLS terms. Moreover, the crops and plant species belonging to the MLS covered by these collections were almost more than 30% of the total plant genera covered by the MLS. They included crops under genera such as *Sorghum*, *Pennisetum*, *Zea*, *Vigna*, *Musa*, *Vicia*, *Phaseolus*, *Solanum*, and a range plant under the genus *Andropogon*. This shows how Sudan could be an important contributing party to the effective functioning and success of this system at the global level. In fact, Sudan has officially notified the ITPGRFA Secretary on 23 September 2010 that 6,351 samples of sorghum, pearl millet, banana and other PGRFA listed in Annex I and maintained in the Plant Genetic Resources Unit of the Agricultural Research Corporation in Wad Medani, Sudan, have been included in the Multilateral System (https://www.fao.org/plant-treaty/areas-of-work/the-multilateral-system/collections). Since then, APGRC has been engaged in exchanging PGRFA with others using the Standard Material Transfer Agreement, which has been set out by the ITPGRFA to regulate the germplasm exchanges within the MLS.

Conservation of collected PGRFA is challenging in Sudan. Arrangements have been taken through an evolutionary process that started in the early 1980s with the establishment of genebanks at central and subnational levels. The seed samples of different accessions have been kept under long-term seed storage conditions in the central seedbank at Wad Medani (14.3931° N, 33.5392° E), with copies of some of them in a subnational seedbank unit in Elobeid Agricultural Research Station at Elobeid (13.1782° N, 30.2167° E) in North Kordofan State. However, the war that erupted in the country in mid-April 2023 and extended to Wad Medani in mid-December 2023 has posed serious threats to seed collections at the APGRC headquarters there. Hence, there is a pressing need to accelerate the duplication of all the materials in the Svalbard Global Seed Vault (SGSV), where only around 3,000 accessions have already been deposited by 2022. If the situation continues to deteriorate due to the ongoing conflict, temporary relocation of the collection from Wad Medani to other safer place(s) should be an option, as experienced by other genebanks in conflict areas. A recent example is the ICARDA genebank, which was originally located in Aleppo, Syria. As a consequence of the Syrian civil war and severe combat operations in Aleppo starting in 2012, the genebank had to be relocated in 2016 to Lebanon and Morocco. Part of the germplasm collection could be restored, with safety duplicates preserved at international genebanks and SGSV, as mentioned by Herbold and Engels (2023) in their study on genebanks risks. Overall, some African and Asian countries were identified as having high political instability, as is currently the situation in Sudan, necessitating the implementation of effective mitigation measures including safety duplication outside the country, which has occurred at low rates so far, and relocation to safer places if the war continues.

Conclusion

The activities for collecting PGRFA from *in situ* habitats in Sudan during the reported period have reasonably succeeded in covering almost all agroecosystems, as well as different farming systems within almost all the Sudan states resulting in a substantial number of accessions from different cultivated crop and plant groups. Among the areas covered were regions that had been affected by armed conflicts in previous years, such as South and West Kordofan States before 2004, and the Darfur region in the far western parts of the country before 2014.

The germplasm collecting activities have successfully gathered a considerable number of accessions from crops for which Sudan is part of the region of diversity and/or origin. Among those were crops such as sorghum, pearl millet, sesame, watermelon, melon, okra and roselle, with each crop having between 200 and more than 2,000 accessions collected.

The collection of local genetic resources of vegetatively propagated crops such as banana and date palm, was initiated for the first time during this period. However, further collecting activities are needed for these crops as well as other vegetatively propagated fruit-producing plants such as mango, guava and citrus.

The genetic resources of indigenous range plants remain a major taxonomic gap, which needs to be filled through extensive germplasm collecting activities. This should include a wide coverage of rangelands across the different ecological zones, including the desert, semi-desert, low-rainfall savannahs on both sandy and clayey soils, as well as high-rainfall savannahs towards the borders with the South Sudan Republic, and the unique mountain vegetation such as in Jebel Marra in the far west. Moreover, crop wild relatives, although relatively covered, still remain poorly represented, and continue to be another major taxonomic gap, including relatives to MLS crops such as sorghum, pearl millet and eggplant, which were somehow sampled during the last years, as well as relatives to non-MLS crops of local importance in Sudan such as melons, watermelon, okra and sesame.

It was interesting to note that accessions were collected in relatively large numbers from about 30% of the genera covered by the MLS of the ITPGRFA, positioninig Sudan, as a party to this treaty, to play a significant role in conserving and providing facilitated access to such PGRFA for use under the MLS terms.

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Supplemental data

- Supplemental Table 1. Germplasm collecting missions across the cultivation seasons from 2002–2003 to 2021–2022.
- Supplemental Material 1. Germplasm Collection Form.
- Supplemental Table 2. Total of collection sites visited by the germplasm collection missions in the different states during the cultivation seasons between 2002 and 2022 and the ranges of coordinates within which they were geographically located.
- Supplemental Table 3. Total accessions collected from each crop or plant and the total number of states from where they were collected.
- Supplemental Figure 1. Total number of states from where total accessions of the genetic resources of different crops collected.
- Supplemental Figure 2. Total number of accessions collected from different crop wild relatives.
- Supplemental Figure 3. Total number of accessions collected from each range plant species.

Author contributions

I.M. El Tahir: Leading germplasm collection planning and coordination, proposing the paper, writing the first draft, revising and finalizing the manuscript.

A.Z. Babiker, E.A. Abdalla, A.A.E. Ahmed: Participation in planning, leading some collection missions and revising manuscript drafts.

M.O.Y. Goda: Participation in planning, leading documentation process for all collection data and revising manuscript drafts.

M.A.M. Elgabri: Participation in planning, assisting in leading some collection missions, and revising manuscript drafts

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Morphological and biochemical characterization of Ethiopian mustard (*Brassica carinata* A. Braun) germplasm grown in Central Ethiopia

Ermias Estifanos [*,a], **Tileye Feyissa** [b], **Kassahun Tesfaye** [c], **Alemu Lencho** [a] **and Christina Eynck** [d]

[a] *Department of Plant Science, Ambo University, Guder, Ethiopia*
[b] *Institute of Biotechnology, Addis Ababa University, Addis Ababa, Ethiopia*
[c] *Bio and Emerging Technology Institute, Addis Ababa, Ethiopia*
[d] *Saskatoon Research and Development Centre, Agriculture and Agri-Food Canada, Saskatoon, SK, Canada*

Abstract: Ethiopian mustard (*Brassica carinata* A. Braun) shows potential for diverse applications, including as leafy greens, green manure and oilseed feedstock for biofuels. This study evaluated the seed and oil production potential and phenotypic diversity of 49 *B. carinata* accessions through trials conducted in 2018 at the Holeta and Asela Research Centers in Ethiopia, using a lattice design. Data were collected on phenological, morphological, agronomic and seed quality traits. The analysis revealed significant variability across most traits, except for silique width and oil and protein content at Asela, and main raceme length and total glucosinolate content at Holeta. Combined analysis showed significant genotype-by-location interactions for flowering date, seeds per silique and seed yield per hectare, indicating a strong environmental influence on these traits. Phenotypic and genotypic correlation analyses identified strong positive correlations between leaf traits and phenology, seed yield and seed quality, while oil content was negatively associated with protein and glucosinolate content. Principal component analysis identified five components at Asela and six components at Holeta with eigenvalues greater than one, explaining over 77% of the total variation at both locations. Key traits such as plant height, seed yield and oil content contributed significantly to these principal components. Cluster analysis grouped the accessions into three clusters based on distinct trait combinations. Accessions 17545, 21373, 24203 and 24495 consistently performed well across multiple traits across sites, making them strong candidates for breeding programmes focused on improving seed yield and quality in *B. carinata*.

Keywords: *Brassica carinata*, germplasm characterization, oil content, seed yield

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Introduction

Brassica carinata A. Braun (Ethiopian mustard, carinata) (BBCC) known as 'Gomenzer' in Amharic, a natural amphidiploid hybrid between *B. nigra* (BB) and *B. oleracea* (CC), is believed to have originated in the Ethiopian highlands (Warwick and Black, 1991). It is well adapted to these highlands (Prakash and Hinata, 1980; Rakow, 2004) and to a wider range of climatic conditions (Montemurro *et al*, 2016). The crop is grown globally for diverse applications. In the Horn of Africa, it is primarily utilized as leafy greens and as oilseed for culinary use (Basili and Rossi, 2018; Hagos *et al*, 2020), while in Europe it is employed for green manure and biofuel production (Cosentino *et al*, 2008; Montemurro *et al*, 2016). In North America, *B. carinata* is grown for biofuel and oleochemical applications (Blackshaw *et al*,

*Corresponding author: Ermias Estifanos (ermiasestifo@yahoo.com)

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2011; Marillia *et al*, 2014; Gesch *et al*, 2015; Lawton, 2019; Seepaul *et al*, 2021; Nóia-Júnior *et al*, 2022), whereas in Asia it is primarily cultivated for oilseed production (Katiyar *et al*, 1986; Lawton, 2019; Thakur *et al*, 2019).

B. carinata seeds are rich in oil, with contents ranging from 25% to 47% by weight (Taylor *et al*, 2010; Redda *et al*, 2022; Ambaw *et al*, 2024a). The oil is used as an edible oil in Ethiopia after being blended with Nuge (*Guizotia abyssinica*) oil and/or other vegetable oils (Belete *et al*, 2012; Bosekeng, 2019; Thakur *et al*, 2019; Alemaw and Gurmu, 2023). Characterized by a high erucic acid content, the oil possesses desirable physicochemical properties, such as low free fatty acid content and abundant unsaponifiable matter, enhancing its stability and quality (Cardone *et al*, 2003; Mohdaly and Ramadan, 2022). These attributes, combined with the low moisture and acidity levels ($<$ 1% and 0.98% as oleic acid, respectively), make *B. carinata* oil a versatile feedstock. Its industrial applications encompass biodiesel and bio-jet fuel production, as well as the manufacturing of lubricants, plastics, soaps and detergents (Mohdaly and Ramadan, 2022; Redda *et al*, 2022). The biomass, particularly its high-protein content seed meal, serves as a nutritious supplement for livestock, poultry and swine (Paula *et al*, 2019; George *et al*, 2021). It also helps manage soil-borne pests through biofumigation, releasing biocidal compounds, particularly isothiocyanates, which are effective against various soil pathogens (dos Santos *et al*, 2021; Iboyi *et al*, 2022).

Studies on *B. carinata* revealed a complex interplay between genetic diversity, phenotypic variation and agronomic potential. Population structure analyses using SNP (Tesfaye *et al*, 2023) and SSR markers (Thakur *et al*, 2020; Zhou *et al*, 2022) indicated moderate genetic diversity, low heterozygosity and limited gene flow. Tesfaye *et al* (2023) reported heterozygosity of 0.30 and nucleotide diversity of 1.31 $\times$ 10^{-5}, while Thakur *et al* (2020) observed an average of 3.03 alleles per marker. Phenotypic studies highlighted significant morphological diversity, including oil content (Mohdaly and Ramadan, 2022; Ambaw *et al*, 2024b). *B. carinata* also demonstrated superior productivity, economic returns and environmental performance compared to other mustard species (Rathore *et al*, 2022). Further research has explored trait correlations and identified markers for seed quality (Zhang *et al*, 2017), and QTL for traits like flowering time and oil content (Mohdaly and Ramadan, 2022). Characterizing germplasm is essential for understanding phenotypic and genetic diversity, and is crucial for selecting accessions for breeding programmes and designing conservation strategies (Kumari *et al*, 2023; Salgotra and Chauhan, 2023).

Morphological traits, such as seed size and plant height, are primary criteria for selecting accessions in breeding programmes due to their ease of measurement (El-Esawi, 2018; Swarup *et al*, 2021). Traits such as seed oil content and quality, as well as biomass production, help to select genotypes meeting standards for consumption or industrial use (Zhang *et al*, 2017; Tesfaye *et al*, 2019).

Some studies have assessed several morphological traits of *B. carinata*, such as plant height, leaf shape, seed size and flowering time. Zada *et al* (2013) evaluated 132 accessions and documented significant phenotypic variability. Hagos *et al* (2020) also reported significant differences in leaf size attributes ($p < 0.001$) and phenological traits of 313 accessions.

Despite extensive research on the potential of Ethiopian mustard for biofuel applications, a comprehensive understanding of its morphological, agronomic, seed and oil content traits remains crucial for developing effective breeding strategies for a range of purposes, as these traits have a significant impact on seed and oil production. This study aims to evaluate *B. carinata* accessions to determine their seed and oil production potential and diversity to be utilized in breeding programmes.

Materials and methods

Plant material

A total of 49 Ethiopian mustard accessions were evaluated in this study. The planting materials comprised 47 landraces, randomly selected from eight geographically clustered regions within the collections of the Ethiopian Biodiversity Institute, Addis Ababa Ethiopian Biodiversity Institute (EBI, https://ebi.gov.et). Additionally, two released varieties, 'Holetta-1' and 'S-67', developed by the Holeta Agricultural Research Center, were included. The passport data of the accessions used and the pedigree of the released varieties are provided in Supplemental Table 1.

The Ethiopian Biodiversity Institute (EBI), the source of the landrace accessions, provided limited quantities of seed for each accession. Specifically, each accession was provided with only 4g of seed, which was insufficient for establishing trials at more than two locations. This limitation restricts the assessment of genotype-by-environment (G$\times$E) interactions across a wider range of environmental conditions.

Experimental site

Field experiments were conducted at two agricultural research centres in Ethiopia during the 2018 cropping season. The first location, Holeta, is situated 40km southwest of Addis Ababa at an altitude of 2,400m above sea level (m.a.s.l.), with coordinates of 9°3′N latitude and 38°30′E longitude. The soils in this area are predominantly nitosols and vertisols with the region receiving an average annual rainfall of 1,008.14mm (Tura *et al*, 2021) and a mean maximum and minimum temperature of 22.3°C and 6.15°C, respectively (Geleta *et al*, 2024). The second location, Asela, is located 170km south of Addis Ababa at an altitude of 2,200 m.a.s.l., with coordinates of 8°1′N latitude and 39°9′E longitude. Asela's soils are primarily

lateritic and black cotton, with an average annual rainfall of approximately 1,000mm (ETWRDEC (2017); Fekadu (2021)) and a mean minimum and maximum temperature of 5.0°C and 28.0°C, respectively (Megersa *et al*, 2024).

Experimental procedure

Experiments were conducted at two sites utilizing a 7×7 lattice design with two replications of 49 accessions. The design was chosen due to the limited seed availability from EBI. The plot size was 1.8m^2, consisting of four rows 30cm apart and measuring 1.5m in length. Seeds were sown by hand drilling in rows at a rate of 5kg/ha. At both sites, fertilizers at a rate of 46kg/ha nitrogen and 69kg/ha P_2O_5 were applied. Following EARO (2004) guidelines, conventional agricultural practices, encompassing field preparation, nutrient application and weed control measures were implemented.

Data collection

Plant-based data: Measurements were conducted from five randomly selected plants per plot. Leaf petiole length, leaf length and leaf width were measured from five middle leaves of each selected plant. Number of leaves per plant, plant height, number of primary branches per plant and seed yield per plant were recorded from the same five plants. Main raceme length and number of siliques per main raceme were recorded from five main racemes of the same selected plants. Silique length, silique width, and number of seeds per silique were measured for five siliques selected from the main racemes of each of the same five plants. Mean values for the measured traits were calculated from these measurements. Measurements of selected traits were conducted according to the International Board for Plant Genetic Resources descriptors for *Brassica* and *Raphanus* (IBPGR, 1990).

Plot-based data: For each plot, the following data were collected from the two central rows: days to flowering, days to maturity, thousand seed weight and seed yield per hectare.

Seed quality analysis was conducted at the Agriculture and Agri-Food Canada – Saskatoon Research and Development Centre in Saskatchewan, Canada. The oil and protein content of the seeds was determined using near-infrared reflectance spectroscopy (Foss Model 6500). Oil and protein concentrations were expressed as percentages on a moisture-free basis (Hossain *et al*, 2018). Total glucosinolates were extracted and purified using ion-exchange chromatography and on-column enzymatic desulfation (Thies, 1980). Trimethylsilyl derivatives were prepared using the acetone and 1-methylimidazole-based method of Landerouin *et al* (1987).

In this study, data were collected at various growth stages according to the Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH) scale. Early leaf development traits were recorded at BBCH 19, branching and flowering traits at BBCH 50, and silique, seed, and maturity traits at BBCH 90 (Meier, 2018).

Data Analysis

Data analysis was performed using R software (R Core Team, 2023) packages. Analysis of variance (ANOVA) was conducted using the 'PBIB.test' function from the agricolae package (Mendoza, 2023) for individual location analyses, while the 'aov' function from the same package was used for the combined analysis across locations. Scatter plot mean seed yield per hectare comparison was performed by R software ggplot2 package (Wickham, 2016). Mean separation was carried out using Tukey's Honestly Significant Difference (HSD) test, also implemented in the agricolae package (Mendoza, 2023). Before doing the combined analysis of variance, a test for homogeneity of variance was performed (F-test) (Mead *et al*, 2003). The phenotypic and genotypic correlation coefficients were calculated using the R software variability package (Popat *et al*, 2020), which implements the methods described by Singh and Chaudhary (1977) as described below:

Phenotypic correlation coefficient:

$$r_p = \frac{Cov_{p(xy)}}{\sqrt{V_{p(x)} V_{p(y)}}}$$

Where $Cov_{p(xy)}$ is the phenotypic covariance between trait x and trait y, $V_{p(x)}$ and $V_{p(y)}$ are the phenotypic variances of x and y, respectively.

Genotypic correlation coefficient:

$$r_g = \frac{Cov_{g(xy)}}{\sqrt{V_{g(x)} V_{g(y)}}}$$

Where $Cov_{g(xy)}$ is the genotypic covariance between trait x and trait y, $V_{g(x)}$ and $V_{g(y)}$ are the genotypic variances of x and y, respectively.

Heatmaps of the correlation results were created by the R software pheatmap package (Kolde, 2019). Principal component analysis (PCA) and Ward's D^2 Linkage and Euclidean distance hierarchical method cluster analysis were performed using the 'factoextra' (Kassambara and Mundt, 2020) and 'FactoMineR' (Le *et al*, 2008) packages. The optimal number of clusters was determined using 'NbClust' package (Charrad *et al*, 2014).

Results and discussion

Overview of trait variation across locations

Accessions showed coefficients of variation (CV) ranging from 4.8% to 32.8% (Table 1), consistent with the high coefficients of variation (> 20) observed for traits such as leaf petiole length, leaf length, leaf width, primary branches per plant, number of siliques per main raceme, and seed yield per hectare at both locations in this study. Ambaw *et al* (2024b) also reported similarly high CVs, suggesting that these traits exhibit substantial phenotypic plasticity and/or are strongly influenced by genetic factors segregating within our accessions (Table 1). The fact that high CVs were observed at both locations implies a consistent influence, hinting at significant G×E interactions. The average seed yield per hectare was slightly higher at Asela, at

Table 1. Summary statistics of agronomic, morphological, and quality traits for 49 *Brassica carinata* accessions evaluated at Asela and Holeta. LPL, leaf petiole length (cm); LL, leaf length (cm); LW, leaf width (cm); LPP, leaves per plant; DF, days to flowering; DM, days to maturity; PH, plant height (cm); PB, primary branches per plant; MRL, main raceme length (cm); SPMR, number of siliques per main raceme; SL, silique length (mm); SW, silique width (mm); SPS, number of seeds per silique; TSW, thousand seed weight (g); SYppl, seed yield per plant (g); SYpha, seed yield per hectare (kg); OIL, oil content (%); PRO, protein content (%); TG, total glucosinolate content (μmol/g); Min, minimum; Max, maximum; SD, standard deviation; CV, coefficients of variation.

Trait	Asela				Holeta			
	Mean ± SD	Min	Max	CV	Mean ± SD	Min	Max	CV
LPL	13.5 ± 3.7	4.9	21.7	27.6	11.4 ± 3.7	2.7	19.5	32.8
LL	28.2 ± 6.5	9	40.7	23.1	26.3 ± 6.5	7	38.2	24.5
LW	10.9 ± 2.2	5.8	15.8	19.9	12.4 ± 2.5	5.4	18.5	20.1
LPP	99 ± 14.2	67	123.1	14.3	92.9 ± 14.4	59.5	118.1	15.5
FD	86.7 ± 10.4	61	109	12	92 ± 9.7	66	114.5	10.5
MD	153.1 ± 14.5	127	180.5	9.5	174.4 ± 8.4	148.5	188.5	4.8
PH	197.7 ± 13.9	162	220.5	7	188.7 ± 19.2	114	219.5	10.2
PB	14.3 ± 3	8.2	20.3	21.2	14.5 ± 3.1	8	20.3	21.2
MRL	5.5 ± 0.6	4.5	6.6	10.8	5.2 ± 0.4	4.4	6.1	8
SPMR	18.4 ± 4.5	11.2	28.6	24.6	18.6 ± 5.1	10	32.8	27.3
SL	28.5 ± 4.4	19.8	38.6	15.5	30.3 ± 4.9	20.3	40.2	16
SW	2.2 ± 0.2	1.7	2.8	10.6	2.4 ± 0.2	2.1	3.1	8.4
SPS	12.8 ± 1.4	9.8	15.6	11.1	12.7 ± 1.5	9.5	15.6	12.2
TSW	3 ± 0.5	1.9	4	16	3.1 ± 0.5	2.2	4.1	14.8
SYppl	3.6 ± 0.8	2.2	5.2	22.6	3.1 ± 0.8	1.6	4.7	26.2
SYpha	2,245.4 ± 500.3	1,262.1	3,074.2	22.3	2,161.6 ± 500	1,173.9	2,950.5	23.1
OIL	37.2 ± 2.5	32.6	43.1	6.7	44 ± 2.2	37.4	49.1	5
PRO	29.9 ± 2.1	25.8	34.6	7.1	26 ± 1.8	22.7	31.5	6.8
TG	99.9 ± 9.7	82.6	123.3	9.7	83.3 ± 8.5	61.6	99.2	10.2

2,245.4 ± 500.3kg (ranging from 1,262.1 to 3,074.2kg), compared to 2,161.6 ± 500kg at Holeta (ranging from 1,173.9 to 2,950.5kg) (Table 1). This slight increase in mean yield at Asela, along with consistent standard deviations across locations, indicates relative similarity in environmental conditions. However, the average oil content was higher at Holeta, at 44 ± 2.2% (ranging from 37.4 to 49.1%), compared to 37.2 ± 2.5% (ranging from 32.6 to 43.1%) at Asela. The difference in trend between seed yield and oil content may be attributed to environmental differences, while the broad range in yields and location-based variation in oil content highlights the genotypic variability of *B. carinata*, offering valuable potential for selection in future crop improvement efforts.

Comparable yields for *B. carinata* have been reported in other studies. Seepaul *et al* (2021), in a literature review of multiple field trials, summarized yields ranging from 1,929kg/ha to 2,732kg/ha. Furthermore, Tesfaye *et al* (2023) found a seed yield average of 3,185kg/ha on selected *B. carinata* genotypes in Ethiopia. Similarly, Iboyi *et al* (2023) reported a range from a minimum seeding rate of 732kg/ha at a 1.12kg/ha to a maximum seeding rate of 1,087kg/ha at 5.6kg/hain southeastern United States. For oil content, previous studies on *B. carinata* in Ethiopia have also reported comparable findings. Ambaw *et al* (2024b) reported oil content values ranging from 37.88% to 46.98%, while Redda *et al* (2022) observed oil content between 35% and 45% in Ethiopian germplasm. These results are consistent with the findings of this study, highlighting the rich phenotypic diversity and potential for improving seed yield and oil quality in *B. carinata*.

Analysis of variance

Individual location analysis results showed significant variability among tested *B. carinata* accessions for most of the traits except silique width, oil and protein content at Asela and main raceme length and total glucosinolate content at Holeta (Table 2). Combined location analysis of variance revealed significant variation among accessions for all traits, while the interaction between accession and location showed significant variation for the traits flowering date, number of seeds per silique and seed yield per hectare (Table 3).

The significant variations observed in most studied traits among *B. carinata* accessions indicate substantial genetic diversity within the species. This aligns with Tesfaye *et al* (2023), who reported that 93% of the genetic variation was attributed to molecular variance, and Thakur *et al* (2020), who found an average genetic diversity of 0.37. This diversity provides opportunities for breeding programmes to develop cultivars with desirable traits. The significant interaction between genotype and location for certain traits suggests that the performance of *B. carinata* accessions is influenced by environmental factors. This implies that

Table 2. Analysis of variance mean squares for replication, genotype, block, and error across agronomic, morphological, and quality traits for 49 *B. carinata* accessions evaluated at Asela and Holeta. LPL, leaf petiole length (cm); LL, leaf length (cm); LW, leaf width (cm); LPP, leaves per plant; DF, days to flowering; DM, days to maturity; PH, plant height (cm); PB, primary branches per plant; MRL, main raceme length (cm); SPMR, number of siliques per main raceme; SL, silique length (mm); SW, silique width (mm); SPS, number of seeds per silique; TSW, thousand seed weight (g); SYppl, seed yield per plant (g); SYpha, seed yield per hectare (kg); OIL, oil content (%); PRO, protein content (%); TG, total glucosinolate content (μmol/g); CV, coefficients of variation. **, and *, denote significance at $p \leq 0.01$, and $p \leq 0.05$ respectively.

Trait	Asela					Holeta				
	Replication	Genotype	Block	Error	CV (%)	Replication	Genotype	Block	Error	CV (%)
LPL	0.98	27.7**	0.5	0.5	5.2	56.94**	27.7**	0.6	0.5	6.5
LL	169.28**	84.8**	20.7	20.4	16.0	379.7**	83.4**	19.2	21.1	17.4
LW	44.72**	9.5**	2.7	2.1	13.4	31.09**	12.4**	3.2	2.5	12.7
LPP	263.19**	403.4**	10.8	13.5	3.7	26.13	416.7**	12.9	14.8	4.1
FD	32	217**	6.7	7.9	3.2	54.38**	186.1**	11.1*	4.7	2.4
MD	2.3	420.4**	51.1	43.9	4.3	3.31	142.3**	35	23.3	2.8
PH	1,389.4**	387.3**	84.6	141.6	6.0	11,100.5**	733.3**	120.8	153.3	6.6
PB	0.09	18.5**	2.2	2.9	11.8	266.48**	18.9**	3.3	3	11.9
MRL	0.5	0.7**	0.4	0.2	8.8	71.54**	0.4	0.2	0.3	10.1
SPMR	5.93	40.8**	2.8	3.1	9.6	6.9	51.5**	2.3	5.4	12.5
SL	4.57	39**	18.6	17.2	14.5	188.3**	47.2**	23.6	18.2	14.1
SW	0.06	0.1	0.1	0.1	14.4	0.01	0.1**	0.1	0.04	8.0
SPS	0.03	4**	1.6	1.4	9.4	0.74	4.7**	0.8	1.5	9.7
TSW	0.11	0.4**	0.1	0.1	11.9	0.29	0.4**	0.1	0.1	11.6
SYppl	0	1.3**	0.1	0.1	7.3	0.17	1.3**	0.04	0.1	7.9
SYpha	5,437.06	500,587.4**	30,678.6	28,324.7	7.5	134,332.25*	499,973.6**	35,619.7	18,572.4	6.3
OIL	19.2	12.5	7.4	9.7	8.4	0.52	9.5**	3.9	3.2	4.1
PRO	29.85	9.1	3.4	6.1	8.3	15.35*	6.3*	5.2	3.7	7.4
TG	710.02**	186.6**	56.4	84.2	9.2	1,142.64**	145.1	137.2	110.5	12.6

Table 3. Analysis of variance mean squares for location, genotype and their interaction across agronomic, morphological and quality traits in the combined evaluation of 49 *Brassica carinata* accessions. LPL, leaf petiole length (cm); LL, leaf length (cm); LW, leaf width (cm); LPP, leaves per plant; DF, days to flowering; DM, days to maturity; PH, plant height (cm); PB, primary branches per plant; MRL, main raceme length (cm); SPMR, number of siliques per main raceme; SL, silique length (mm); SW, silique width (mm); SPS, number of seeds per silique; TSW, thousand seed weight (g); SYppl, seed yield per plant (g); SYpha, seed yield per hectare (kg); OIL, oil content (%); PRO, protein content (%); TG, total glucosinolate content (μmol/g); CV, coefficients of variation. **, and *, denote significance at $p \leq 0.01$, and $p \leq 0.05$ respectively.

Trait	Location	Genotype	(G×L)	Error	CV (%)
LPL	223.93**	55.4**	0.02	1.1	8.5
LL	180.13**	168.1**	0.04	25.7	18.6
LW	107.86**	21.6**	0.3	3.2	15.3
LPP	1,836.73**	818.5**	1.6	16.2	4.2
FD	1,363.72**	391.6**	11.5*	7.7	3.1
PB	2.17	37.2**	0.2	5.5	16.3
SPMR	2.87	90.3**	2	3.9	10.6
SL	167.15**	83.9**	2.3	20.1	15.3
SW	3.12**	0.13**	0.06	0.07	11.3
SPS	0.75	6.4**	2.4*	1.4	9.3
TSW	0.62*	0.8**	0	0.1	11.2
SYppl	13.31**	2.5**	0.1	0.1	7.7
SYpha	344,348.37**	948,487.3**	52,073.7**	26,771.9	7.4
OIL	14,693,939.17**	99,738**	44,010.9	38,121.2	11.7
PRO	765.71**	9.4**	6.1	5.1	8.1
TG	13,474.13**	206.2**	125.5	114.1	11.7

selecting genotypes for specific growing conditions is crucial for optimal yield and quality. The lack of significant variation in silique width and oil and protein content at Asela (Table 2) might indicate a relatively narrow genetic variation or a strong genetic control over these traits; however, in the case of silique width, although the genotypic mean square was similar between Asela and Holeta, significance was detected only at Holeta due to its lower experimental error, which increased the F-ratio and statistical power to reveal genotypic differences. Meanwhile, the significant variation in main raceme length and total glucosinolate content recorded at Asela but not at Holeta (Table 2) suggests that there is a high environmental influence on the expression of these traits. The significant interactions between genotype and location for flowering date, seeds per silique and seed yield per hectare highlight the importance of considering both genetic factors and environmental conditions when selecting genotypes for specific regions.

Similar significant variability among *B. carinata* genotypes has been reported by Belete (2011); Walle *et al* (2014); Dhaliwal *et al* (2019); Amsalu (2020b,c); Mahendra-Salam *et al* (2021) and Khan *et al* (2022). The observation that the majority of genotype-by-location (G×L) interactions in our combined analysis of variance were non-significant (Table 3) aligns with the findings of Adeniji and Aloyce (2012), but contrasts with the reports of significant G×L interactions by Kumar *et al* (2020) and Tadesse *et al* (2021). This discrepancy in results could stem from several factors related to the experimental design, the germplasm studied and the environmental conditions.

Correlation analyses

Phenotypic correlation analysis at the two locations revealed significant positive and negative associations among various agronomic and seed traits (Figures 1 and 2). Leaf and phenological traits exhibited the strongest positive phenotypic correlations at both locations, including leaf petiole length with leaf length (0.80, 0.80), leaf width (0.80, 0.78), flowering date (0.78, 0.72), and maturity date (0.73, 0.58); leaf length with leaf width (0.90, 0.89), flowering date (0.72, 0.70), and maturity date (0.65, 0.58); leaf width with flowering date (0.70, 0.66) and maturity date (0.64, 0.61); and flowering date with maturity date (0.81, 0.79). Additionally, strong positive phenotypic correlations were observed for seed yield per plant with seed yield per hectare (0.87, 0.92) and protein content with total glucosinolate content (0.81, 0.80) at Asela and Holeta, respectively. Similarly, consistently significant negative phenotypic correlations were recorded for oil content with protein content (-0.83, -0.78) and with total glucosinolate content (-0.80, -0.52) at Asela and Holeta, respectively.

To fully understand the genetic relationships underlying the observed phenotypic variation, analyzing genotypic correlations among the studied traits is essential. This analysis offers valuable insights into the genetic linkages between traits, aiding in the identification of potential breeding targets and providing a deeper understanding of the genetic limitations within *B. carinata*.

The genotypic correlation analyses of this study revealed several significant associations among the considered traits for both locations (Figures 1 and 2). Strong positive correlations were again observed among leaf and phenological traits, including leaf petiole length with leaf length (0.99, 0.99), leaf width (0.98, 0.92), flowering date (0.80, 0.77) and maturity date (0.81, 0.70); leaf length with leaf width (0.98, 0.96), flowering date (0.89, 0.98) and maturity date (0.91, 0.99); and leaf width with flowering date (0.82, 0.89) and maturity date (0.88, 0.98). Flowering date also showed high correlations with maturity date (0.92, 0.97). Additionally, strong positive genotypic correlations were recorded for seed yield per plant with seed yield per hectare (0.99, 0.99) and plant height (0.61, 0.58); and for thousand seed weight with leaf length (0.67, 0.72), leaf width (0.77, 0.78), maturity date (0.57, 0.61), and plant height (0.72, 0.66) at Asela and Holeta, respectively. Seed yield per hectare showed significant positive associations with the number of siliques per main raceme (0.52, 0.52) and thousand seed weight (0.53, 0.49). Negative significant correlations were recorded for oil content with protein content (-0.50, -0.66) and total glucosinolate content (-0.84, -0.59) at Asela and Holeta, respectively.

The strong positive correlations observed in this study between leaf traits (leaf length, leaf width, and leaf petiole length) are consistent with previous research by Yimer *et al* (2021). This suggests a consistent genetic and environmental influence on these traits in *B. carinata*. As expected, the positive correlation between plant height and phenological traits (flowering and maturity dates) observed in this study aligns with Walle *et al* (2014). This relationship is likely due to the natural elongation of the flowering spike contributing to plant height. However, confirming this correlation in *B. carinata* under the studied conditions helps strengthen its role in breeding for synchronized flowering and uniform plant architecture.

The positive correlations between seed yield per hectare and seed yield per plant are further supported by Amsalu (2020a) and Belete (2011). These findings highlight the importance of seed yield per plant and the number of siliques per main raceme as key determinants of overall yield. Marjanović-Jeromela *et al* (2007) provide additional evidence for the significance of these traits in Brassica species. The positive correlation of thousand seed weight with various traits, including leaf length and width, maturity date and plant height, aligns with the findings of Tadessel and Alemu (2019). This underscores the importance of seed weight in yield improvement.

The inverse relationship between oil content and protein and glucosinolate contents observed in this study is consistent with previous research by Tadessel

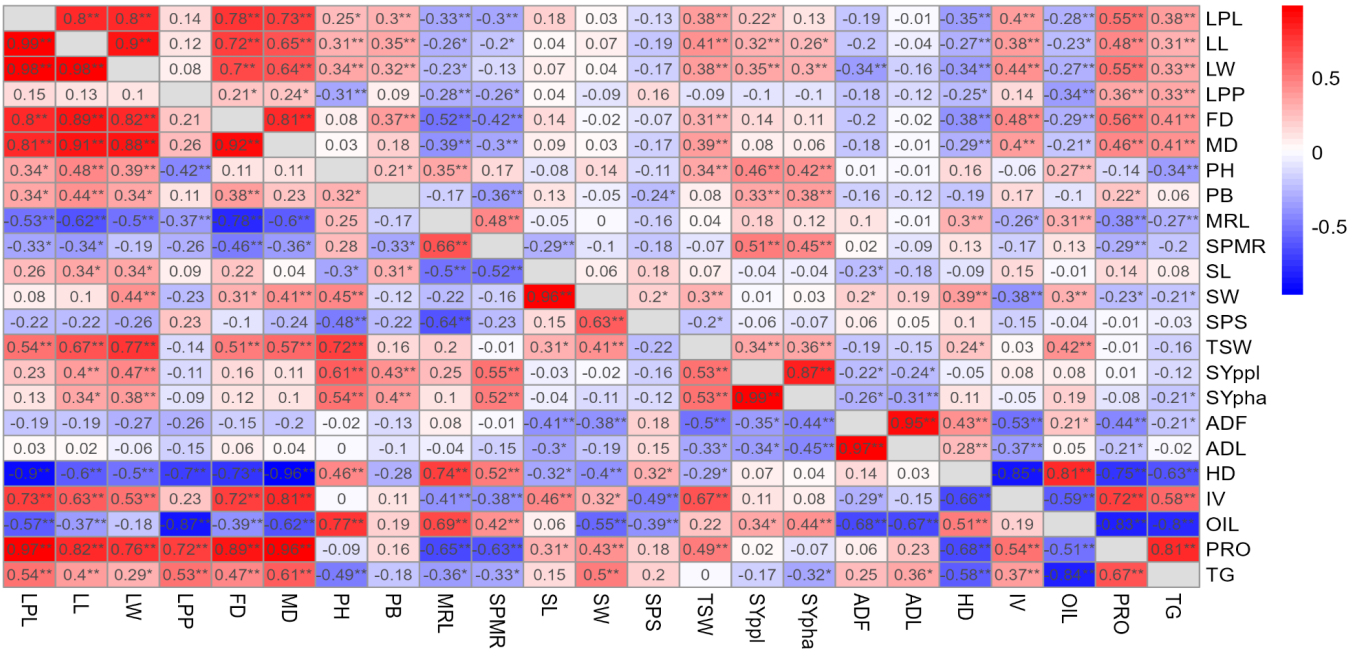

Figure 1. Heatmap displaying phenotypic (above diagonal) and genotypic (below diagonal) correlation coefficients for 19 traits measured in *Brassica carinata* accessions grown at Asela. LPL, leaf petiole length (cm); LL, leaf length (cm); LW, leaf width (cm); LPP, leaves per plant; DF, days to flowering; DM, days to maturity; PH, plant height (cm); PB, primary branches per plant; MRL, main raceme length (cm); SPMR, number of siliques per main raceme; SL, silique length (mm); SW, silique width (mm); SPS, number of seeds per silique; TSW, thousand seed weight (g); SYppl, seed yield per plant (g); SYpha, seed yield per hectare (kg); OIL, oil content (%); PRO, protein content (%); TG, total glucosinolate content (μmol/g). **, and *, indicates significant at $p \leq 0.01$, and $p \leq 0.05$, respectively.

and Alemu (2019), confirming the common trade-off between these seed quality traits in oilseed crops.

However, the positive genotypic correlations between thousand seed weight and phenological traits in this study differ from the negative associations reported by Khan *et al* (2022) and Kumar-Singh *et al* (2018). Different *B. carinata* accessions possess distinct genetic architectures (Tesfaye *et al*, 2023). Discrepancies in correlation results may be partly attributed to the simple lattice design employed. Residual environmental heterogeneity within blocks, location-by-block interactions and incomplete blocking effects could have influenced trait correlations. Future studies should consider spatial analysis techniques to account for these factors. The observed inconsistencies in the correlations between main raceme length and protein and oil content, as well as silique width and total glucosinolate content, across different locations, suggest that environmental factors play a significant role in influencing the expression of these traits. This result highlights the need for future research that specifically investigates the G×E interactions governing these complex trait relationships in *B. carinata*.

The strong associations observed between leaf, phenological and seed traits in this study highlight their potential as target traits for breeding programmes aimed at improving *B. carinata* yield and quality. However, the influence of environmental factors on trait expression underscores the importance of conducting multi-location trials and considering genotype–environment interactions when selecting genotypes for specific regions.

Principal component analysis

A principal component analysis (PCA) was employed to examine the combined data from Asela and Holeta. This analysis identified six principal components (PCs), each with eigenvalues greater than 1 according to Kaiser's criterion (Jolliffe, 2002). Together, these six PCs accounted for 82.0% of the total variability (Supplemental Table 2). The first two components (PC1 and PC2) represented 52.7% of the overall variation and were visualized in a biplot (Supplemental Table 2, Figure 3). The distribution of accessions on the biplot reveals considerable genetic diversity among the accessions. PC1, accounting for 31% of the variance, is characterized by strong positive loadings for leaf petiole length (0.90), leaf length (0.94), leaf width (0.92), days to flowering (0.89), and days to maturity (0.88), indicating that accessions with greater values for traits that influence plant structure and phenology grouped together in PC1 and suggests that selecting for plant leaf area could lead to later maturity. PC2, which explained a further 21.7% of the variation, was characterized by substantial contributions from plant height (0.69), seed yield per plant (0.73), seed yield per hectare (0.75) and the number of siliques per main raceme (0.61), implying a connection between taller plants with a

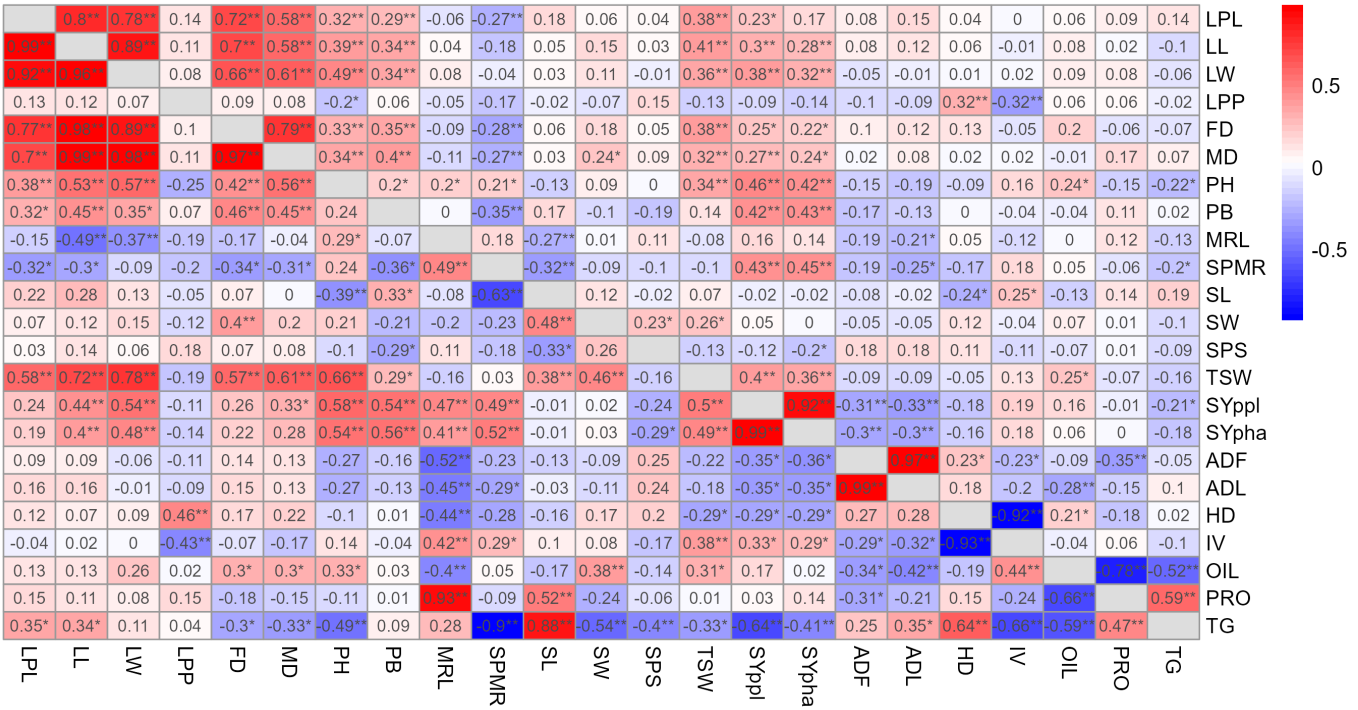

Figure 2. Heatmap displaying phenotypic (above diagonal) and genotypic (below diagonal) correlation coefficients for 19 traits measured in *Brassica carinata* accessions grown at Holeta. LPL, leaf petiole length (cm); LL, leaf length (cm); LW, leaf width (cm); LPP, leaves per plant; DF, days to flowering; DM, days to maturity; PH, plant height (cm); PB, primary branches per plant; MRL, main raceme length (cm); SPMR, number of siliques per main raceme; SL, silique length (mm); SW, silique width (mm); SPS, number of seeds per silique; TSW, thousand seed weight (g); SYppl, seed yield per plant (g); SYpha, seed yield per hectare (kg); OIL, oil content (%); PRO, protein content (%); TG, total glucosinolate content (μmol/g). **, and *, indicates significant at $p \leq 0.01$, and $p \leq 0.05$, respectively.

prolific density of siliques that led to high seed yield. This association points to a potential breeding target: increasing plant height and silique density to enhance overall seed yield. However, it is crucial to consider the potential for lodging associated with taller plants and high silique density. Breeding efforts should also focus on improving stem strength and lodging resistance to ensure that increased yield is not compromised by plant instability.

The distribution of accessions on the biplot reveals considerable genetic diversity, suggesting a rich resource for future breeding efforts and highlighting the potential for selecting lines with desirable trait combinations. The PCA biplot demonstrated that accessions 24494, 24495, 21373, 24203 and 17545 were closely aligned with traits such as plant height, seed yield per plant, seed yield per hectare and thousand seed weight, all of which exhibited strong positive loadings on PC2. This close relationship suggests that these accessions are strongly associated with increased plant height and seed yield potential. Similarly, accessions 21383, 208404, 212665, 19959 and 208412 were positioned near the oil content (Figure 3), demonstrating the potential for enhanced seed oil production. In contrast, the released varieties 'S-67' and 'H1' were not best suited for seed-related and oil traits.

Abraha *et al* (2024) used PCA for the classification of 313 *B. carinata* accessions for 18 traits and reported the first and second PCs accounted for 34.3% of the observed variability. PC1 explained 22.2% of the variability between the morphological attributes and most strongly accounted for the differences between the accessions, which is a lower proportion than this study PC1 variation (31.03%). The difference may be attributed to differences in the genetic diversity and population structure of the germplasms analyzed. Belete (2011) analyzed 36 *B. carinata* accessions for nine agro-morphological traits and reported that 91.4% of the total variation was contributed by the first five principal components, which is a comparable result to the 82.03% cumulative variance explained by the first six PCs in our study. Kumar *et al* (2020) also applied principal component analysis using seven traits of 11 *B. carinata* accessions and found that only the first four principal components showed eigenvalues greater than one and they cumulatively explained a similar proportion (82.46%) of the total variability in this study. This suggests that all three datasets capture a large proportion of the total variability within a relatively small number of underlying PCs. The closeness in variability proportion suggests that, despite the differences in sample size and specific traits considered, the patterns of diversity within *B.*

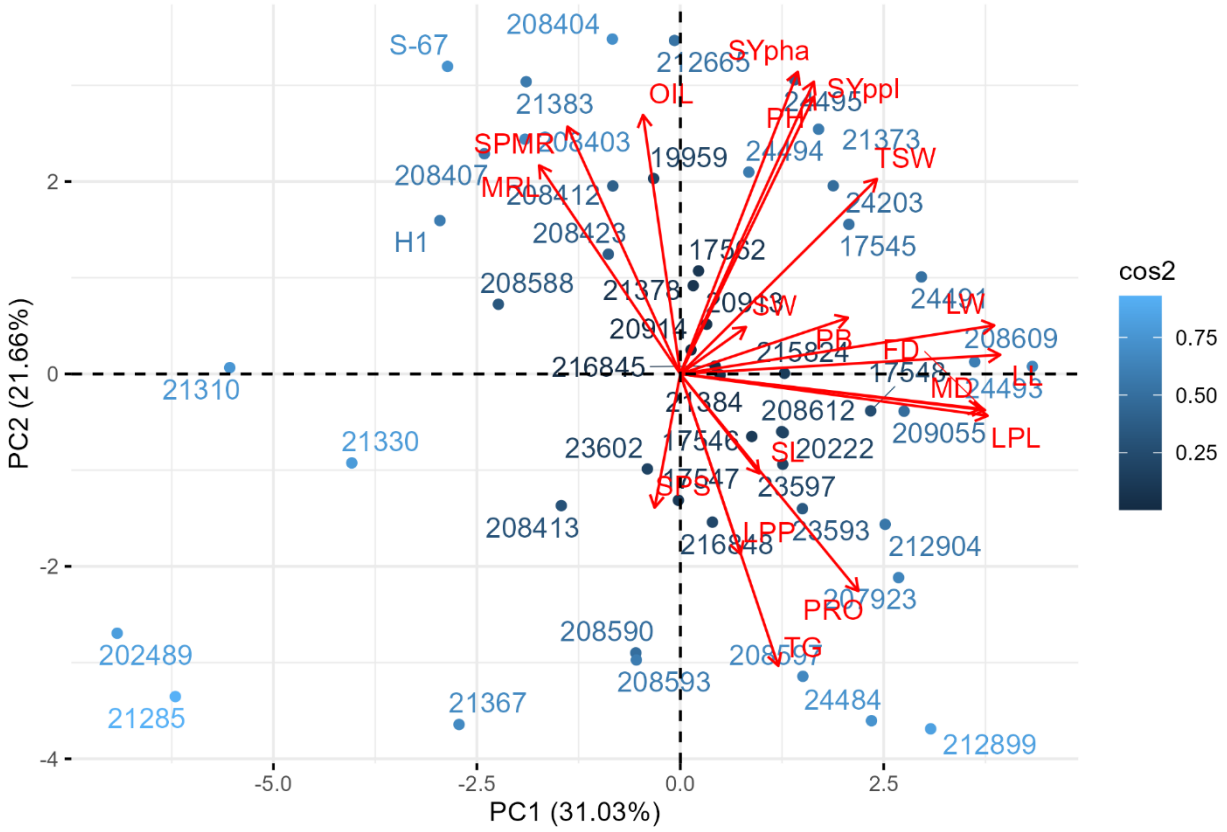

Figure 3. CA biplot of the first two principal components of *B. carinata* accessions, highlighting trait loadings (red arrows) and accession distribution (blue dots) across combined locations. LPL, leaf petiole length (cm); LL, leaf length (cm); LW, leaf width (cm); LPP, leaves per plant; DF, days to flowering; DM, days to maturity; PH, plant height (cm); PB, primary branches per plant; MRL, main raceme length (cm); SPMR, number of siliques per main raceme; SL, silique length (mm); SW, silique width (mm); SPS, number of seeds per silique; TSW, thousand seed weight (g); SYppl, seed yield per plant (g); SYpha, seed yield per hectare (kg); OIL, oil content (%); PRO, protein content (%); TG, total glucosinolate content (μmol/g). Direction of red arrow indicates the association of the corresponding trait with the principal components, while the length of the arrow reflects the magnitude of the trait's contribution (longer arrows = stronger influence); light blue accessions with higher $\cos^2$ values (well-represented by PC1 and PC2); deep blue, accessions with lower $\cos^2$ values (poorly represented by PC1 and PC2).

carinata germplasms are consistently captured by a relatively small number of PCs. These PCA results hold significant agricultural implications for informing *B. carinata* breeding programmes aimed at enhancing seed yield, oil content and overall agronomic performance.

Cluster analysis

Cluster analysis can be applied to various samples and descriptors to examine the relationships and distances among them. In germplasm collections, it is useful for assessing genetic similarities and differences. Understanding genetic distance, the measure of dissimilarity between accessions, is key to predicting the success of crop improvement efforts (Peeters and Martinelli, 1989).

Cluster analysis conducted on the combined data from Asela and Holeta grouped the tested accessions into three distinct clusters of 21, 21, and 7 members, respectively (Figure 4). The two released varieties were both placed in Cluster I. Cluster I is characterized by the highest mean scores for seed yield per hectare, seed yield per plant, plant height and main raceme length (Supplemental Table 3). Cluster II is distinguished by the highest mean values for leaf petiole length, leaf length, leaf width, number of leaves per plant, days to flowering, days to maturity, number of primary branches per plant, silique length, protein content and total glucosinolate content. Cluster III has the highest mean values for number of siliques per main raceme, number of seeds per silique and seed oil content. Cluster III exhibited the highest intra-cluster distance (5.6), indicating a high level of genetic diversity among the accessions (Supplemental Table 3). The greatest inter-cluster distance was recorded between Cluster II and Cluster III (7.4), which suggests a high level of genetic divergence, making accessions from these clusters promising for hybridization. This was followed by the distance between Cluster I and Cluster III (7.3), and between Cluster I and Cluster II (6.0).

The clustering results from the dendrogram corresponded to the findings of both principal component analysis (PCA) and correlation studies. For instance,

accessions 17545, 17562, 24203, 21373, 24494, 24495, 212665, 20913 and 24491 fall in the same quadrant of the PCA biplot and are closely associated with plant height, seed yield per plant, seed yield per hectare, thousand seed weight, number of siliques per main raceme, primary branches per plant, leaf length and leaf width; these traits are significantly positively correlated with seed yield per hectare. The primary distinctions between clusters are influenced by the same characteristics that are significant contributors to the first and second principal components. Accessions grouped within the same cluster exhibited greater similarity to each other than those in separate clusters, further confirming the relationships identified through these analytical methods.

The diversity in cluster numbers identified across *B. carinata* research, can be attributed to variations in genetic diversity, accession selection and study design. For example, the lower cluster counts reported by Muthoni (2010) (two clusters in 47 genotypes) and Adeniji and Aloyce (2012) (three clusters in 14 genotypes)are likely due to smaller sample sizes and limited genetic representation. Conversely, studies with larger numbers of more diverse accessions, such as those by Zada *et al* (2013), Abraha *et al* (2024) (eight clusters in 313 genotypes) and Ambaw *et al* (2024a) (seven clusters in 386 genotypes), captured greater genetic diversity by incorporating genotypes from multiple geographic regions, agroecological zones and possibly wild relatives.

Our study, which identified three distinct clusters among 47 *B. carinata* landraces, aligns with the trend observed in these previous studies. The number of clusters we found is consistent with studies that utilized a moderate sample size and landrace accessions, which are known to exhibit substantial genetic diversity. This suggests that while our sample size was comparable to Muthoni (2010), the inclusion of diverse landraces contributed to the identification of a greater number of distinct groups.

These disparities also highlight the impact of methodological choices: more recent studies employing advanced genotyping tools (e.g. SNPs or SSRs) and sensitive clustering algorithms (e.g. STRUCTURE) have enabled a more precise analysis of population structure. In our study, we used phenotypic data for clustering, which, while informative, may not have captured the same level of genetic resolution as molecular markers. However, the congruence between our cluster analysis, PCA, and correlation studies suggests that the phenotypic data effectively revealed meaningful genetic relationships among the accessions.

The variability in cluster numbers underlines the need for standardized experimental approaches that integrate extensive, diverse germplasm collections with high-resolution markers to enhance genetic diversity, refine breeding strategies and preserve adaptive alleles in *B. carinata*.

Mean performance comparison

Mean seed yield per hectare values for the evaluated accessions showed a clear positive relationship between Asela and Holeta (Supplemental Figure 1). This finding is consistent with studies like Abu *et al* (2022), who used the Additive Main Effects and Multiplicative Interaction (AMMI) analysis to identify six *B. carinata* accessions exhibiting relatively stable performance across environments. As mean yield increased at Asela, it also tended to increase at Holeta, indicating that accessions with high seed yield per hectare in one location generally performed well in the other.

Tukey's mean difference test was applied to traits that showed significant results in the analysis of variance. Based on mean performance accessions 17545, 21373, 21378, 24203, 24493, 24494, 208609, 212665 and 216845 exhibited increased leaf length, increased leaf width, a greater number of primary branches, longer siliques, more seeds per silique, higher thousand seed weight, higher seed yield per hectare, higher oil content, higher protein content and a high total glucosinolate content at both locations (Supplemental Tables 4 and 5, respectively).

However, there were two exceptions: accession 21378 did not rank first in terms of the number of primary branches per plant at either location, and accession 24493 did not rank first for total glucosinolate content at Asela with respect to the traits under consideration. Despite these exceptions, the identified accessions showed strong potential for improving key traits like seed yield, oil content and protein content, all of which are economically valuable. The variability in rankings for 21378 and 24493 accessions with regard to specific traits suggests that these accessions may exhibit trait-specific responses depending on the environment.

In this study, genotypic variation was assessed based on phenotypic differences in the studied traits. Significant variations among the *B. carinata* accessions were observed, which is consistent with the findings of Tesfaye *et al* (2023), who reported moderate genetic diversity in *B. carinata* populations (average expected heterozygosity = 0.31, polymorphism information content = 0.26), indicating variability among accessions. While our study assessed phenotypic diversity based on mean trait values, Thakur *et al* (2020) reported a gene diversity of 0.37, which reflects the average genetic variation within populations. Khedikar *et al* (2020) reported low molecular genetic diversity in *B. carinata* accessions (heterozygosity = 0.30, nucleotide diversity = 1.31×10^{-5}), suggesting limited variability due to a narrow genetic base and potential inbreeding effects. Phenotypic diversity reflects the combined effects of genetic and environmental factors, while molecular diversity captures variation at the DNA level. Differences in sampling strategies, molecular markers and potential inbreeding effects can influence molecular genetic diversity estimates and contribute to discrepancies between studies. It is important to acknowledge that direct comparisons between phenotypic and

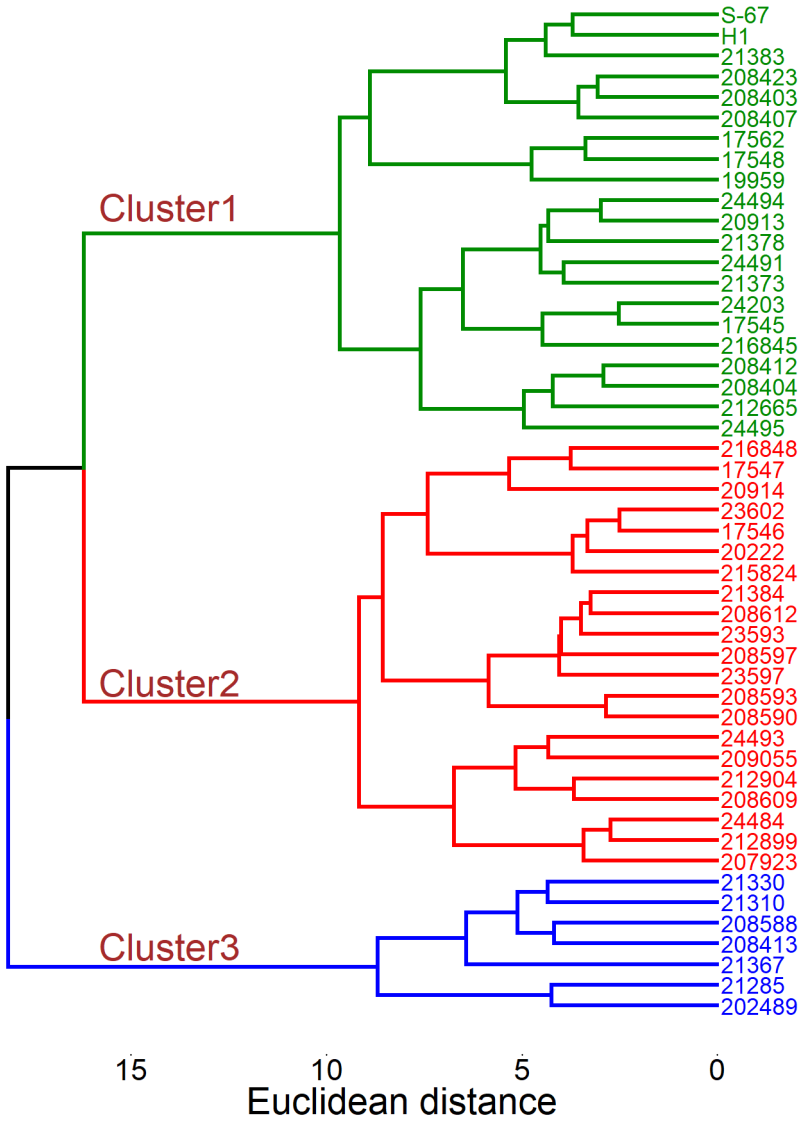

Figure 4. Dendrogram of 49 *Brassica carinata* accessions assessed for 19 studied traits at Asela and Holeta using Ward's D2 Linkage and Euclidean distance.

molecular diversity estimates should be interpreted with caution, as they reflect different aspects of genetic variation. These differences in genetic diversity estimates could be attributed to variations in the *B. carinata* populations studied, the types of molecular markers used and the sampling strategies employed. Further research is needed to elucidate the underlying factors contributing to these discrepancies.

Positive correlations between seed yield per plant and siliques per main raceme suggest opportunities for enhancing overall yield (Dwivedi *et al*, 2023; Saini *et al*, 2023). PCA effectively identified key traits: plant height, seed yield per plant, seed yield per hectare, thousand seed weight and oil content driving variation within the *B. carinata* accessions. This helps in pinpointing promising genotypes for crop improvement, as the initial principal components capture a significant percentage of the total variance, focusing on genetic differences that influence important agronomic traits like seed and oil yield.

Moreover, clustering accessions based on trait similarities supports targeted breeding strategies. However, the variability in genotype rankings across different environments highlights the importance of considering G×E interactions in breeding programmes (Kumar *et al*, 2020; Tesfaye *et al*, 2024). Thus, these findings underscore the need for robust multi-location and multi-season trials to effectively evaluate genotype adaptability and optimize crop improvement efforts for *B. carinata*. While our study revealed variability in accession rankings across the two locations, suggesting potential G×E interactions, the limited number of experimental sites (Haleta and Asela) restricts our ability to draw strong conclusions. As detailed in the Materials and Methods, seed limitations from EBI constrained our study to these two locations. Consequently, the observed variation may reflect random environmental

noise rather than robust G×E patterns. Future studies should prioritize multi-location trials to capture a wider range of environmental influences and provide more reliable insights into genotype adaptability.

Conclusion

The study emphasizes the importance of genetic diversity within *B. carinata* and the need for multi-location trials to ensure the identification of accessions with stable performance and adaptability across different environments. However, due to limited seed availability from EBI, this study was restricted to two experimental locations, limiting the scope of G×E interaction analysis. Based on our findings, crop improvement efforts should prioritize accessions 24494, 24495, 21373, 24203 and 17545 for yield improvement (based on plant height, seed yield per plant, seed yield per hectare, and thousand seed weight) and accessions 21383, 208404, 212665, 19959 and 208412 for enhanced oil content (based on plant height, seed yield per plant, seed yield per hectare, and oil content). To maximize trait expression, especially for environmentally influenced traits like flowering date and seed yield, location-specific selection is crucial. Additionally, conducting multi-location trials is essential for understanding genotype adaptability and ensuring consistent performance across diverse environments, ultimately supporting more effective crop improvement strategies for *B. carinata*.

Our cluster analysis aligns with the trend of moderate cluster numbers. However, the use of phenotypic data for clustering, rather than high-resolution molecular markers, may have limited the precision of our genetic diversity assessment. Future studies should integrate advanced genotyping tools and diverse germplasm collections to enhance the understanding of genetic diversity and refine breeding strategies in *B. carinata*.

Supplemental data

Supplemental Table 1. Passport data of the accessions used and pedigree information of the released varieties

Supplemental Table 2. Loadings, eigenvalues, and variances of principal components with eigenvalues greater than one from PCA of 49 *Brassica carinata* accessions at Asela and Holeta

Supplemental Table 3. Cluster mean values and intra- and inter-cluster distances for 49 *Brassica carinata* accessions at Asela and Holeta

Supplemental Table 4. Mean performance evaluation results for 19 traits of 49 *Brassica carinata* accessions at Asela

Supplemental Table 5. Mean performance evaluation results for 19 traits of 49 *Brassica carinata* accessions at Holeta

Supplemental Figure 1. Mean Seed yield per hectare (SYpha, in kg) comparison of 49 *Brassica carinata* accessions in two locations: Asela vs. Holeta

Author Contributions

Ermias Estifanos was responsible for material preparation, data collection, analysis, and drafting the first version of the manuscript. Kassahun Tesfaye contributed to the study conception and design. Tileye Feyissa supervised the study and provided feedback on previous versions of the manuscript. Alemu Lencho facilitated the research process. Christina Eynck conducted the seed analysis, provided resources and provided comments on earlier drafts of the manuscript. All authors read and approved the final manuscript.

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Conflict of interest statement

The authors have no relevant financial or non-financial interests to disclose.

Data availability

The raw datasets generated and/or analyzed during the current study are not publicly available as they have not been uploaded to a data repository but are available from the corresponding author upon reasonable request.

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Across borders – the status and future opportunities for long-term conservation of Nordic animal genetic resources

Ellen-Louisa Fagerheim White[a], Maria Kjetså[b], Jaana Peippo[a], Lucy Morgan[b], Juha Kantanen[c], Pierre Comizzoli[d], Lise Lykke Steffensen[a], Morten Kargo[e], Tullis Matson[b], Ian Mayer[f] and Mervi Honkatukia[*,a]

[a] *The Nordic Genetic Resource Center (NordGen), Alnarp, Sweden*
[b] *UK National Livestock Biobank, Whitchurch, Shropshire, United Kingdom*
[c] *Natural Resources Institute Finland (LUKE), Jokioinen, Finland*
[d] *Smithsonian's National Zoo and Conservation Biology Institute, Washington DC, United States of America*
[e] *Center for Quantitative Genetics and Genomics, Aarhus University, Aarhus, Denmark*
[f] *Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Ås, Norway*

Abstract: The genetic diversity of multiple animal species is now declining rapidly, highlighting the need for action to protect and preserve animal genetic resources for the long term. The Nordic countries house a broad range of farm and companion animal breeds and subspecies that play a critical role in environmental sustainability, food safety and security, and human activities. Unfortunately, close to 80% of these breeds and subspecies are either endangered or critically endangered, with population sizes too small to ensure their long-term survival. In addition, almost half of them have either a declining or unknown demographic trend, and many of them suffer from high inbreeding. Emerging pressures such as climate change, infectious diseases and public unrest further threaten the status of the populations, and urgent action is necessary to ensure their future survival. Consequently, efforts for safeguarding the genetic diversity of animal genetic resources (AnGR) with additional *in vitro* or cryoconservation efforts need further consideration. The Nordic conservation strategies for AnGR have traditionally been based on *in vivo* or live conservation. Although cryoconservation efforts are in place for some species, the number of donors and doses varies considerably between breeds and species. Due to the increasing demand for additional measures for safeguarding AnGR, this document discusses the status of active AnGR conservation measures in the Nordic countries and emphasize the central role of regional cooperation in ensuring AnGR sustainability and long-term viability. Further, the contributions of cryoconservation in mitigating genetic losses are discussed.

Keywords: *In vivo* conservation, live conservation, *in vitro* conservation, genebank, cryoconservation, Nordic food security

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Background

The global decline in biodiversity also includes our farm animal breeds

There is a growing global concern about the continued loss of animal biodiversity. Evidence suggests that we have entered the "sixth mass extinction"; the first in Earth's history to be driven primarily by human

[*]Corresponding author: Mervi Honkatukia (mervi.honkatukia@nordgen.org)

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activity (Cowie *et al*, 2022; WWF, 2022). Currently, species are disappearing 10 to 1,000 times faster than the normal 'background' rate of extinction, and the number of individuals in multiple species is declining rapidly (WWF, 2022; IUCN, 2024). While most attention has focused on wildlife, the conservation of the world's numerous native breeds of farm animals, and the necessity to maintain genetic diversity within these species also need attention.

The Food and Agriculture Organization of the UN (FAO) published the *Global Plan of Action for Animal Genetic Resources* in 2007 (FAO, 2007b) and the *First State of the World's Animal Genetic Resources for Food and Agriculture* (FAO, 2007a). This was followed up in 2015, when FAO published the *Second Report on the State of the World's Animal Genetic Resources for Food and Agriculture* (FAO, 2015), to further highlight the importance of the need for additional measures under the Global Plan of Action. The world's commitment to the Global Plan of Action was reaffirmed in 2017 by the 16th Regular Session of the Commission on Genetic Resources for Food and Agriculture and the 40th FAO Conference. A third edition of the Global Plan of Action for Animal Genetic Resources is currently in preparation. Despite the various implemented conservation efforts and the continuously increased awareness of the many important roles of native animal genetic resources (e.g. genetically, environmentally, economically and culturally), 6.7% of the worlds native breeds have become extinct, and 26% are classified as being at risk of extinction (FAO, 2019). The loss of heritage breeds in current production systems may have a detrimental impact on efforts to improve future animal production systems (FAO, 2007a; Kantanen *et al*, 2015).

The Nordic commitment to conservation of animal genetic resources

The Nordic countries – Denmark (including Greenland), Finland (including Åland), Iceland, Norway, Sweden, and the Faroe Islands – have a rich and diverse assemblage of local and regional transboundary farm animal breeds and subspecies (Kierkegaard *et al*, 2020; White *et al*, 2024c). These animals hold strong cultural and historical importance, and both livestock species and companion animals like sheep, goats, cattle, horses, dogs and cats have a long history of association with human societies (Ovaska *et al*, 2021; Bläuer, 2024; Kroløkke *et al*, 2024). The ancestors of some of the breeds even go back as far as 3,000–2,000 BCE, and several of them were important during the Viking age (Bläuer, 2015). Thus, these animals represent an invaluable part of the socioeconomic history of the Nordic countries. Notably, the grazing animals also play an essential role in ecosystem biodiversity and sustainability (Bele *et al*, 2018; Hall, 2018; Fraser *et al*, 2022). Unfortunately, at present, many of the local breeds face the risk of extinction due to dimishing population sizes, highlighting the urgent need to implement additional conservation measures before it is too late (FAO, 2007a; White *et al*, 2024c).

Importantly, the Nordic countries are committed to the "conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of benefits arising from genetic resources" as stated in the 1992 UN Convention on Biological Diversity (UN, 1992). This commitment encompasses both wild and domestic animals and is further outlined for domesticated animals in the FAO Global Plan of Action for Animal Genetic Resources (FAO, 2007a), highlighting their responsibility to safeguard the Nordic native breeds.

Additional approaches are needed

While the conservation of Nordic animal genetic resources (AnGR) has primarily focused on maintaining live populations (*in vivo* conservation), national efforts for cryoconservation of e.g., sperm, embryos and somatic cell tissue (*in vitro* conservation) of AnGR have also been carried out in all the Nordic countries. However, current and future threats such as emerging diseases and climate change, combined with small populations with high inbreeding levels, highlight the importance of additional safeguarding for the breeds. Plant safeguarding initiatives, including backup repositories in the Millennium Seed Bank[1] for wild plants in the UK and the Global Seed Vault in Svalbard[2] for cultivated crops have been successfully operating for some time. However, there have not yet been significant regional efforts for farm animal cryoconservation in the Nordics. The Nordic network project titled 'Nordic animal genebanks - added value through Nordic cooperation' (NordFrost, 2021–2024; White *et al* (2024a)), aimed to strengthen the collaboration and competence for cryopreservation of AnGR in the Nordic region. NordFrost gathered stakeholders in the Nordic countries to discuss the possibilities and challenges of genebanking in the region (White *et al*, 2024a). Concerns on the costs versus benefits of cryoconservation were some of the topics that were raised as obstacles influencing genebanking activities. However, new methods for collection and preservation of material have made cryoconservation and utilization of genetic resources more cost-effective, allowing a wider range of stakeholders to participate in cryoconservation (Blackburn *et al*, 2023; FAO, 2023). Many Nordic countries are now recognizing the need to improve cryoconservation activities in combination with continued efforts for live conservation (for example, the National Strategies of the Nordic Countries, Supplemental Material 1).

[1] https://www.kew.org/wakehurst/whats-at-wakehurst/millennium-seed-bank
[2] https://www.croptrust.org/work/svalbard-global-seed-vault/

Aims and objectives

This paper is a result of the work from the NordFrost network on finding new possibilities for optimized cryoconservation of Nordic AnGR. It aims to advocate for active conservation measures that emphasize the central role of regional cooperation and adaptation of new techniques in ensuring the sustainability and long-term viability of AnGR in the Nordic countries. Thus, the status, challenges, opportunities and future possibilities of cryoconservation in the Nordic countries are presented.

Animal genetic resources in the Nordic countries and conservation strategies

External threats to genetic diversity in animal populations from Nordic countries

The Nordic native breeds face a range of external challenges such as economic and political unrest (e.g. economic fluctuations, warfare influencing prices and availability of feed), modern farming practices (e.g. intensification and industrialization), societal acceptance, climate change, and emerging diseases (FAO, 2007a; Clasen *et al*, 2020, 2021; White *et al*, 2024c,b). The effect of climate change, including global warming and more extreme weather patterns, continues to be of concern as temperatures continues to rise (Simmons, 2022). Overall, the Nordic region is predicted to become both warmer and wetter, and these climatic changes will likely increase the spread and transmission of new disease epidemics threatening both farm animals and wildlife throughout the Nordic region (Gray *et al*, 2009; Wang *et al*, 2011; Yoo *et al*, 2016; Sommer and Cowie, 2020; Ramos *et al*, 2021). Some diseases are already threatening the Nordic farm animal populations. For example, African swine fever (ASF) and highly pathogenic avian influenza (HPAI), infect several populations globally every year (CDC, 2023, 2024). Further, HPAI was reported on 71 fur farms in Finland, causing the euthanasia of nearly half a million animals in 2023 (EFSA *et al*, 2023). The neurodegenerative prion disease classical scrapie has significantly influenced the production of small ruminants in Iceland (Jónmundsson *et al*, 2016; Hauksdóttir, 2021; Thorgeirsdottir, 2022) and recent salmonella outbreaks on poultry farms in Sweden have influenced the national egg supply (SVA, 2024b). More recently, a new strain of vector-borne blue tongue virus has quickly spread through Europe, and has now been detected in Denmark, Sweden and Norway (DEFRA, 2024; Jordbruksverket, 2024; NDCC, 2024; SVA, 2024b,a; Veterinærinstituttet, 2024). Such disease outbreaks can lead to euthanasia of the entire flock of animals on the farm and even on nearby farms to prevent expansion of the epidemic, thereby having critical consequences for the small populations of the local breeds (FAO, 2007a). Some small locally adopted breeds might only exist on one farm or in a small geographical area, which makes them particularly vulnerable.

Internal threats to genetic diversity in animal populations from Nordic countries

Internal threats to animal populations are mainly those of inbreeding and low genetic diversity caused, for example, by a small population size or suboptimal breeding practices. This can lead to the accumulation of deleterious recessive genetic mutations in the population, which, in turn, leads to compromised fertility rates and increased disease rates (Kadri *et al*, 2014). A small population size combined with inadequate breeding strategies can cause loss of genetic variation and lead the population further down in an extinction vortex, as illustrated in Figure 1. Effective management of genetic variation in small populations is therefore critical. Strategies for managing small populations are comprehensively described in *Sustainable Management of Animal Genetic Resources* (Wooliams *et al*, 2005).

Advantages and limitations of live animal conservation and cryoconservation

A population's ability to adapt to its environment and achieve genetic gain is an important factor in both conservation and production populations. Live conservation allows continuous adaptation to a changing environment, and breeding can lead to improved breed performance over time. Cryoconservation is described as lacking the ability for continuous adaptation because the genetics are in a frozen state. Nevertheless, cryoconservation can be seen as an important second layer of safeguarding the populations. Further utilizing genetic variation stored in genebanks may help recover lost traits that are potentially valuable to adapt to new environments and improve performance. Therefore, a combination of live conservation and cryoconservation is the optimal way to go. Further, education regarding cultural heritage traditions, such as landscaping, is important for raising awareness surrounding unique breeds and their history as human companions and production animals. Live conservation can directly promote both tradition and raise awareness of the breeds (FAO, 2013), in a way that cannot be done by cryoconservation alone. However, it can be argued that by storing the genetic material, heritage is also conserved.

Key concerns surrounding the management and conservation of AnGR are related to different pressures, especially those relating to climate change, disease outbreaks and decline in genetic variation. Live conservation in a geographically limited area is especially vulnerable to events such as political unrest, natural disasters and emerging diseases, as well as the decline in genetic variation due to suboptimal breeding practices or too small population sizes. While important tools for inbreeding control, such as optimum contribution selection (OCS) (Grundy *et al*, 2000; Woolliams *et al*, 2015) and biosecurity measures, mitigate genetic loss and the risk of disease, they do not remove the threat completely.

Cryoconservation protects against disease outbreaks and provides the possibility to improve the genetic

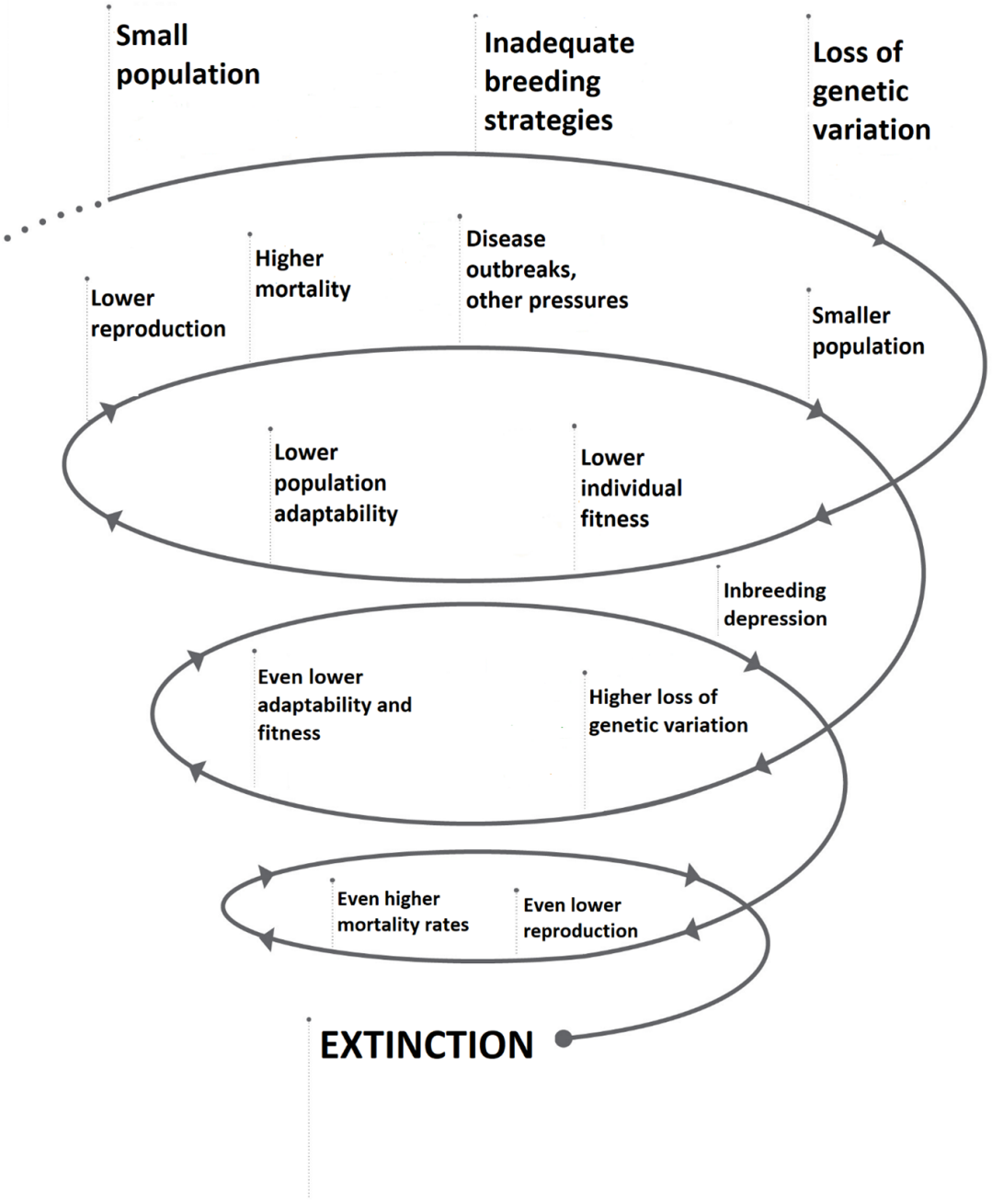


Figure 1. The extinction vortex. The figure above is a simple illustration of the consequences related to the continued loss of genetic variation in an already small population. Loss of variation has a range of driving factors, both related to genetics and inbreeding and human decisions such as breeding strategies, economy and social acceptance.

status of populations that may have suffered large losses. Genetic materials from previous generations can be reintroduced into the populations by using cryopreserved material such as semen (Blackburn *et al*, 2023; FAO, 2023). Implementing cryoconservation combined with live conservation can therefore increase the population carrying capacity, and possibly the effective population size, without influencing the living populations (Eynard *et al*, 2018; Blackburn *et al*, 2023; FAO, 2023). Further, evolving genomic methods and sampling techniques offer tremendous potential for effectively managing the Nordic native breed populations.

Regarding costs, schematics demonstrating the different components needed for establishing a genebank are listed in Figure 2. There is a lack of research evaluating the cost-effectiveness of cryoconservation compared with live conservation. Some evidence suggests that genebanks can be cost-effective: for example, Silversides *et al* (2012) determined that it could be approximately 90% cheaper to conserve chicken genetic resources by storing cryopreserved germplasm as opposed to living populations. However, it is important to consider that

this is an extreme example, only considering genebanking alone. Importantly, it is possible to conserve populations cost-effectively in live populations by developing niche products or creating added value (e.g. animal-assisted therapy, using animals in restoration and management of traditional ecosystems or the production of premium-quality food products).

History, current conservation efforts and risk status

Globally, conservation efforts are still under development or insufficient to ensure the future security of AnGR (FAO, 2015; ERFP, 2021). Meticulous record-keeping and filling the observed gaps in knowledge, such as important genetic parameters and population development, are essential for the success of long-term conservation goals. However, the status of long-term conservation does not only rely on the conservation status of live populations but also on the status of cryoconservation. The Nordic countries have 167 local and regional transboundary farm animal breeds and subspecies. The majority (71%) of the Nordic breeds are endangered or critically endangered, while 5% are considered vulnerable. Only 14% are not at risk, while the remaining 10% have an undetermined risk status (Figure 3; White *et al* (2024c)). Data for the Nordic breeds collected from the Domestic Animal Diversity Information System (DAD-IS) strongly indicate that the numbers of samples stored in national genebanks are not yet sufficient, or representative of the living populations' genetic diversity. FAO has recommended to cryoconserve breeds that are categorized as endangered or critically endangered; however, only 60 of the 119 Nordic breeds with this risk status have cryoconserved materials, and the remaining 59 breeds have no additional safety measures in place (FAO, 2024; White *et al*, 2024c).

The Nordic countries have so far mainly focused on maintaining living populations through live conservation measures. However, all Nordic countries also have some cryoconserved material. The first Nordic cryoconservation efforts included the establishment of semen banks for native cattle breeds in Sweden, Iceland, Denmark and Norway in 1967, 1969, 1971 and 1977, respectively (Maijala, 2011). In Finland, cryopreservation of semen and embryos from the Finncattle breeds began during the 1980s (Pehu *et al*, 2018). The vast majority of the collected material is semen from cattle and sheep breeds (Table 1). The remaining species are highly under-represented in cryoconservation, and no material is stored from any avian species, pigs, cats or bees (Table 1). Cell types that include female genetic material including embryos, oocytes, DNA and somatic cells are also lacking in Nordic cryoconservation according to DAD-IS record of genebanking for conservation.

Number of donors presently used in cryoconservation in the Nordic countries

In addition to having at least some cryoconserved material, the breeds should ideally have sufficient samples preserved to be able to recreate healthy and sustainable future generations (White *et al*, 2024c). Recent species-specific data from the Centre for Genetic Resources, the Netherlands (CGN) of Wageningen University & Research (WUR) evaluates the sufficiency of stored samples according to different criteria. These include the number of donors and samples necessary for future reconstruction of a breed (Van Der Sluis and Schoon, 2024). The general consensus across all species is that a minimum of 50 male donor animals is required to reduce the risk of inbreeding depression. Having 37 donors allow for some selection, but the estimated inbreeding rate per generation will be higher (0.67%), while 25 donor animals yield no possibility for selection because the inbreeding rate per generation will exceed 1%. It is also essential to consider the relationship between the donors for these evaluations (Van Der Sluis and Schoon, 2024).

Data from DAD-IS shows that 19 (24.36%) of the cryoconserved Nordic breeds have sufficient semen doses from enough male donors for population sustainability ($>$ 50 donors) (Figure 4). Furthermore, 7 (9%) of the breeds have cryopreserved embryos. So far, there have been no attempts to preserve somatic cells for genebanking purposes in any country (Table 1, White *et al* (2024c)). Notably, the largest proportion of material cryopreserved is from Norwegian cattle and sheep breeds (Table 1), which also constitute almost half of the breeds with sufficient male donors (Figure 4; FAO (2024); White *et al* (2024c)).

Further, the current collections lack female reproductive material and, in most cases, the genetic characterization of the donor living animals. The scarcity of stored material, combined with the number of breeds that are endangered and critically endangered, highlights the urgent need to improve the efforts to protect and conserve their genetic resources. In addition, the sanitary status of donors collected at artificial insemination (AI) stations is diagnosed according to EU regulations. However, information may be lacking for other genebank collections outside certified collection units (especially for female germplasm). Furthermore, in some cases, current genebanks lack a link to animal pedigree information, genotypes and phenotypes. Utilization of the samples without sufficient information about the sanitary status, pedigree or genotype risks introducing infectious diseases, unintended intensification of breeding, and unwanted genetic defects into the living population later (FAO, 2023). Importantly, utilization of breeding material from genebanks that are not EU-certified also conflicts with EU Animal Health regulations.

Challenges to conservation initiatives in the Nordics

Challenges in collecting and obtaining samples

One of the main reasons why so few species are represented in cryoconservation is that a lot of the stored

The economics of establishing a new genebank

The costs of establishing a new genebank involves two major costings: (1) Initial infrastructure and equipment costs (long-term investment), and (2) Operational costs. Below estimates for the distribution of operational costs are based on the Dutch Gene Bank for Farm Animals managed by the Centre for Genetic Resources, the Netherlands (CGN) of Wageningen University and Research.

1. Initial infrastructure and equipment costs

These costs represent a major part of the budget, and will cover the initial acquisition of suitable premises, including a cryopreservation laboratory and two storage rooms equipped with liquid nitrogen tanks. Purchase of equipment for quality analysis and the processing and freezing of genetic material (microscope, straw-filling machine, programmable freezer). The facility should have monitoring equipment to detect liquid nitrogen spills, as well as a state-of-the-art database system.

2. Operational costs

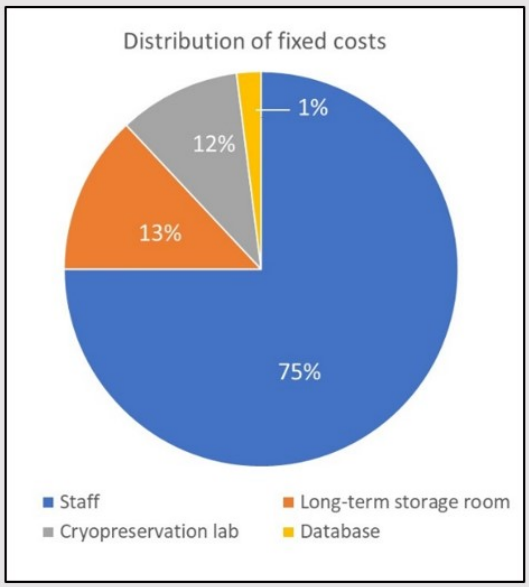
The operational costs consist of a) Fixed costs, and b) Variable costs.

a) Fixed costs

The fixed costs, which represent 80–90% of the operational budget, consist of staff salaries (3-4 positions) as well as rental costs of the cryopreservation lab and the fixed costs associated with storage (liquid nitrogen).

b) Variable costs

The major part of the variable costs is associated with collecting material for the genebank collections. A breakdown of the major costs associated with the fixed and variable costs is shown below.



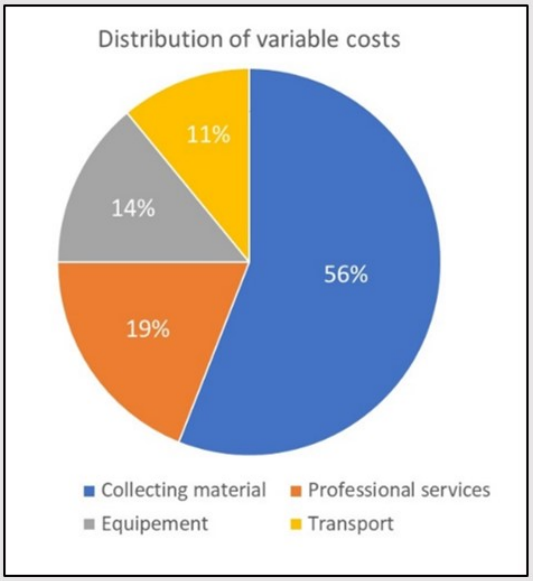


Figure 2. Economic considerations for establishment of a cryoconservation genebank for animal genetic resources. Genebank costs based on the Dutch Gene Bank for Farm Animals.

Table 1. Distribution of cell types of cryopreserved samples between species in the Nordic countries (FAO, 2024). *, Most of the collected DNA and somatic cells until now have been stored to be used in scientific contexts, which is why they have likely not been registered as material that is stored for conservation purposes in DAD-IS.

Species	Number of Breeds	Semen	DNA*	Embryos	Somatic Cells *	Oocytes
Honeybee	0/1	0	0	0	0	0
Cats	0/5	0	0	0	0	0
Cattle	23/28	972,907	190	119	0	0
Chicken	0/21	0	0	0	0	0
Deer	1/1	200	100	1	550	0
Dogs	12/29	1,634	0	0	0	0
Ducks	0/6	0	0	0	0	0
Goats	8/9	21,499	30	4	0	0
Geese	0/6	0	0	0	0	0
Horse	9/14	1,558	34	0	0	0
Pig	2/5	140	0	0	0	0
Pigeon	0/3	0	0	0	0	0
Rabbit	0/5	0	0	0	0	0
Sheep	26/34	75,291	215	108	0	0

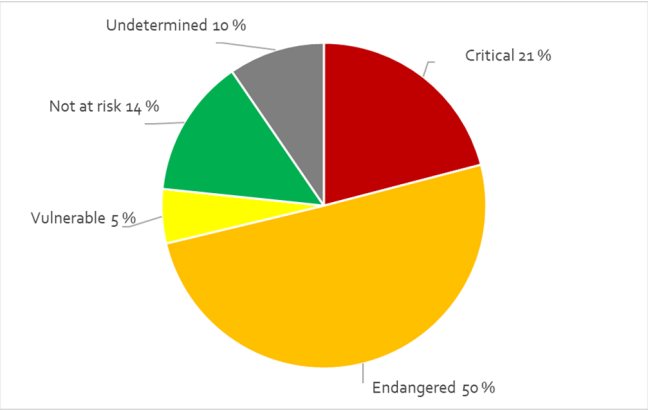


Figure 3. Risk status per breed for the different species of Nordic AnGR. The risk status is based on the classification system from FAO (FAO, 2013). The figure is adapted from White *et al* (2024c).

samples come from species with commercial breeds and are collected in collaboration with commercial breeding partners (e.g. cattle). Sheep, goats, horses and dogs are also species where AI is available, but not as widely used. For some groups of animals, collection of ejaculated semen is also not possible, either because the animals need to be trained beforehand, they are not tame enough, or there are no facilities close by. For avian species, cryopreservation protocols are not yet optimized, which limits the possibility for cryoconservation. Consequently, for example, some poultry are conserved in live genebanks (*ex situ*, *in vivo*) in the Nordics (Brekke, 2017; Sæther *et al*, 2018; Berres *et al*, 2020).

An important challenge is the cryopreservation of female tissue for genebanking. At present, female tissue (oocytes and embryos) is highly underrepresented in the current collections for cryoconservation in the Nordic countries (Table 1). Collection of female reproductive tissues is more expensive than in males (Table 2). However, the cost-effectiveness can be enhanced in species like cattle through hormonal stimulation to induce multiple ovulations. In contrast, for other species, such as horses, even hormonal intervention does not significantly improve efficiency, and collection of even two embryos at a time is rare. Further, the successful use of ovarian tissue as an oocyte reservoir is very limited in domestic animals due to compromised developmental competence of young follicles. Overall, many of the collection techniques require well-established laboratory facilities and staff with sufficient expertise. Thus, establishing sufficient genebanking activities including both male and female tissues within each country can be difficult without enough funding and support. Consequently, there are no collected oocytes at this time, and the cryoconservation of embryos is limited.

Challenges to the establishment and management of long-term genebanks

The most important factors to consider relating to the establishment and management of long-term cryoconservation (genebanking) are long-term storage costs, number of facilities and management tools (e.g. database connecting samples to genotypes and phenotypes), sanitary issues, technical expertise and collection infrastructure, and prerequisites for collaboration (for cross-border cooperation). Cryoconservation is a long-term commitment, and therefore, facility contracts and funding for long-term storage are essential for the management of genebanks (Blackburn *et al*, 2023). In the Nordic countries, nationally cryopreserved genetic material is often stored only in a single location for a given species or breed. Such a form of storage is vulnerable, because it introduces the potential risk of loss of collected material due to unforeseen events such as system malfunction (FAO, 2023). Cryopreserved material is highly valuable and must be safeguarded against poten-

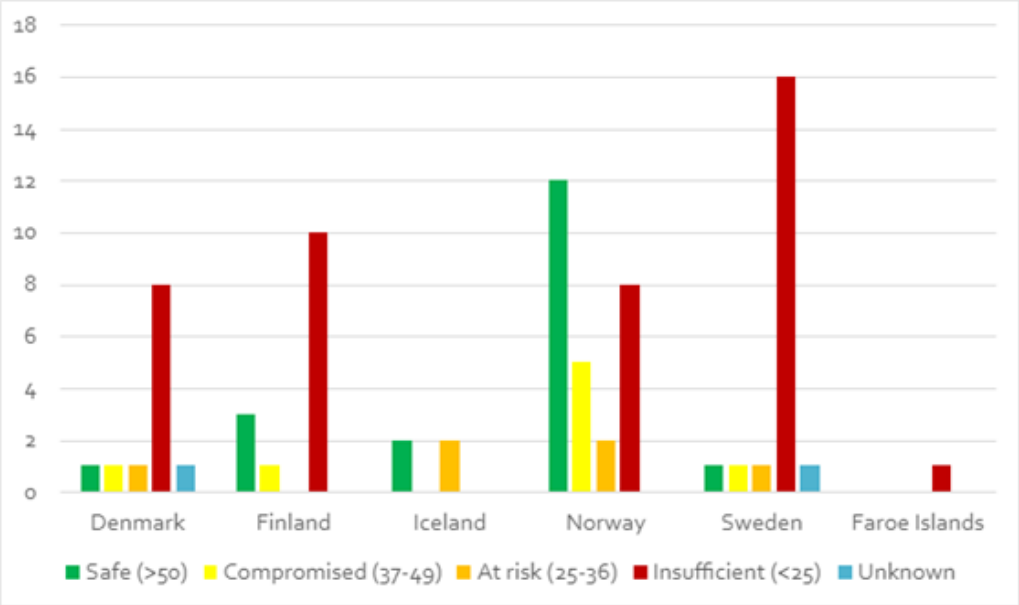


Figure 4. Classification of cryopreserved semen donors from Nordic AnGR breeds based on the criteria developed by the Centre for Genetic Resources, the Netherlands, including the total number of breeds represented in the samples. Total number of breeds with samples: 78 (White *et al*, 2024c).

Table 2. Comparison of cell types used in cryoconservation, and advantages and limitations of chosen sample types

Sample type	Advantages	Limitations
Semen	Cost-effective (large volumes per individual) Optimized protocols (high survival rate) Can be very useful in managing small population (large quantities can be used for sex-sorted semen Well-known sanitary status Can be used to re-create breeds by back-crossing	Expensive collection infrastructure (fewer males used for collection) Collections done by small numbers of centralized systems of commercial companies (due to legislation) Variation in the semen's freezing ability between individuals.
Epididymal sperm	Can be collected from dead and living donors Supports conservation of genetic diversity (i.e., can be collected from more males) Can be used to re-create breeds by back-crossing Cheap infrastructure	Lower quantity per dose Sanitary status must be defined
Oocytes	Easy to collect Can be collected from both live and dead donors (ovum pick-up, sterilization) Collections can be repeated Cheap infrastructure to collect samples from dead individuals Decision upon mating can be postponed to the future	Developmental competence after cryopreservation is very low Collection infrastructure from living donors is expensive Number of oocyte per donor is variable and unpredictable
Early embryos	Good survival rates (depending on origin – in animal or in lab)	Expensive Unpredictable Mating decisions need to be made prior to cryopreservation
Somatic cells (SC) (e.g. skin tissue)	Can be collected from all individuals 100% of DNA Cheap infrastructure for sampling Continuously developing techniques for using SC	Technologies for using SC can be expensive and not always successful
Ovarian and testicular tissues	Contain thousands of gametes at an early stage that can be developed *in vitro*. Genetic value of prepubertal individuals can also be preserved	Collections can be either after sterilization or post-mortem

tial unexpected risks; therefore, it is recommended to set up and maintain at least two separate storage facilities in different geographical locations (FAO, 2023), similar to recommended safety duplication strategies in plant genebanking.

In cases where multi-country collaboration for cryoconservation is contemplated, there are other important factors to consider, such as legislation for cross-border transportation, sanitary requirements (i.e. biosecurity), and the vulnerability to alterations in national laws or disease outbreaks that could influence the retrieval of samples (Blackburn *et al*, 2023). In Europe, EU regulation 807/2014 (EC, 2014) introduced transitional provisions allowing the transport of samples across borders according to certain sanitary regulations. Legislation and requirements for sanitary status can vary according to national legislation and regulations. In cases of collaboration, it has been recommended to implement the strictest regulations for sanitary issues (Blackburn *et al*, 2023). While multi-country collaboration introduces some concerns, it also provides a range of benefits, such as reduced costs and improved expertise. The initial costs of establishing a facility for cryoconservation are often high, which is why many countries only have one national facility where collections are stored. Collaboration between countries could mitigate the risk of malfunction while reducing the cost.

Obtaining the technical expertise necessary for processes such as collecting, freezing and thawing of samples is a key factor for the success of long-term cryoconservation and the possible use of the stored samples in the future. However, knowing the best practices for the collection and cryopreservation steps for each of the different species requires broad expertise. Cross-border collaboration could promote transparency in knowledge and expertise between the countries involved, positively contributing to conservation.

Successful management of both long- and short-term cryoconservation facilities requires the necessary and relevant knowledge for the collected genetic diversity. Blackburn *et al* (2023) argues that establishing a database with this information is not only important for contemporary management, but also for decision-making processes and the evaluation of the genebanking success. Continuous evaluation of genebanks is essential for the future status of overall conservation, because it will highlight their possible strengths and weaknesses.

Future perspectives on AnGR conservation in the Nordic countries

Collaboration across borders

Collaboration and shared knowledge of strategies and infrastructure offer future collective opportunities for the growth and security of Nordic native AnGR. For plants, the Global Seed Vault in Svalbarddemonstrates that collaboration can be highly successful. Further strengthening the collaboration for conservation of AnGR across country borders could be favourable in several ways. For example, exchanging duplicate material on a regional level could reduce the costs of cryoconservation while mitigating the risk posed by system malfunction.

In Europe, an important stakeholder for regional cooperation in cryoconservation for animals is the European Gene Bank Network for Animal Genetic Resources (EUGENA). This network serves as a coordinated digital platform that facilitates the exchange, management and conservation of genetic data from various farm animal species in Europe. One of the main goals of EUGENA is to improve cryoconservation in Europe by promoting transparency of both information and technology among the network's member countries. In this manner, EUGENA promotes genebanking efforts both at a national level as well as at a regional level by supporting countries in fulfilling their individual roles and objectives, and by facilitating cooperation between their European member countries. All European countries, including the Nordics can join the network if their genebanks meet certain criteria and are certified[3]. This network offers immense possibilities for cross-border collaboration.

Further, in the United Kingdom, a proactive group to recognize the need for comprehensive genebanking of farm animals is the UK National Livestock Biobank (UKNLB, https://www.livestockbiobank.com/), which could act as a valuable networking partner providing peer support for the Nordic countries in their initiative and exploitation of the latest cryo-methodologies. Comparable to the Nordic region, up to 80% of the United Kingdom's native farm animal breeds are at risk of extinction, with the loss of such breeds highlighted as a significant threat to national food security (DEFRA, 2021b,a). The UKNLB is a farm animal biobank based on gamete preservation (sperm, egg and embryo), alongside collection and cryopreservation of skin samples for fibroblast cell line generation. The collection of skin samples alongside gametes is unique within the UK farming cryopreservation sector, and has numerous applications and opportunities, including preservation of the whole genetic profile of farm species and the regenerative genetic capture of female lines (see Supplemental Material 2 for more info). Biobanking permits the indefinite storage of genetics beyond the lifespan of the original animal, ensuring the availability of resources for future applications (ERFP, 2023).

Implementing state-of-the-art technologies for future possibilities of conservation

The rapid progress in biotechnology paves the way to a future-oriented approach for genebanks. Anticipated future advancements, including precision gene editing, synthetic biology and advanced reproductive

[3] Memorandum of Understanding (MoU) related to the European Genebank Network for Animal Genetic Resources (EUGENA), accessible from https://www.eugena-erfp.net/en/about/how-to-become-a-member

technologies (Blackburn *et al*, 2023), offer promising avenues (Supplemental Material 2). For example, currently explored methods such as dry-preservation of germinal vesicles offer a simpler and less expensive way for long-term conservation because the preserved material can be stored at room temperature (Graves-Herring *et al*, 2013; Lee *et al*, 2019; Lee and Comizzoli, 2024). Further, advances in sequencing and genomics allow improved characterization of traits as well as better representation of the genetic diversity within genebanks and effective evaluation of populations and inbreeding control. Integrating these technologies into genebank models will enhance the ability to revive and restore endangered populations, ensuring the long-term viability of AnGR.

The NordFrost network has introduced state-of-the-art technologies that have previously been underutilized in traditional genebanking approaches to the Nordic stakeholders, such as epididymal sperm collection and the cryopreservation of skin tissue (Table 2; White *et al* (2024a)). Utilizing epididymal sperm in other disciplines is not a new phenomenon, yet it has not been widely used as a cryoconservation method for AnGR. The advantage of using epididymal sperm is that it can be collected from both deceased and living donors as well as during castration, which means that sperm can be collected from more individuals. This supports the conservation of genetic diversity. Further, like traditionally collected semen samples, it can be used to re-create breeds by back-crossing. For this method, it is important to consider that only one collection event is possible, and subsequently, the number of doses is lower than that of ejaculated semen. Nevertheless, this method is highly valuable for species where routine semen collection poses challenges – e.g. for animals that are difficult to train for semen collection, or in situations where the necessary facilities are unavailable, or where animals die due to unforeseen events and the genetic diversity would otherwise be lost. This method has been successfully implemented in countries like Finland, where epididymal sperm has been frozen from a variety of species, including cattle, horses, sheep, reindeer and roosters.

The cryopreservation of skin tissue also holds significant potential, extending the range of future possibilities for long-term conservation and could act as an additional security measure alongside the traditional sampling of reproductive tissues. Utilizing skin tissue offers an effective and less complicated way of capturing the genetic variation of individuals. For example, where gametes only capture half of the genetic information of the donor animal, skin samples and fibroblast cell lines effectively capture the entire DNA (Gorji *et al*, 2021). Furthermore, while effective infrastructures for cryopreservation and thawing of sperm have been established for most breeds, equally effective procedures for female reproductive tissue have yet to be established (Table 2; Li *et al* (2009)). Consequently, storing skin tissue samples in a genebank overcomes challenges that follow cryopreservation and thawing of egg cells (Li *et al*, 2009). Furthermore, there is also significant progress being made in the development of induced pluripotent stem cell technology, which would facilitate the development of sperm and egg cells from reprogrammed fibroblast cell lines (Bhartiya *et al*, 2014; Horer *et al*, 2023). The prospect of this technology is substantial as it would allow for the creation of individuals with a fresh genetic set, from a readily taken and cryopreserved skin sample. Most development so far has been in lab-based mice, with pups already born from reprogrammed skin samples (Mahabadi *et al*, 2018; Moradi *et al*, 2019). More detailed descriptions of the increased recognition and utilization of cryopreserved skin tissue in conservation can be explored in Supplemental Material 2.

Final remarks

To date, the Nordic countries have maintained high sanitary standards for animal health. However, maintaining these standards is expected to become increasingly challenging. Recent outbreaks of blue tongue, HPAI and African swine fever spreading to the Nordics highlight the importance of additional safeguarding measures for the conservation of animal genetic resources. Cryoconservation is a possibility to add a second layer of conservation measures and safeguard animal genetic resources, as it protects against disease outbreaks and provides the possibility to improve the genetic status of populations that may have suffered large losses due, for example, to disease epidemics.

One of the main goals for conservation in the Nordic countries is to maintain healthy and stable populations, which implies maintenance of genetic variation within the populations. Implementing both live conservation and cryoconservation allows for continuous adaptation and promotion of cultural heritage, while maintaining and/or improving genetic variation, and protecting against disease outbreaks that are difficult to contain. Therefore, the combination of both live conservation and cryoconservation enhances the safeguarding of both current and future AnGR (FAO, 2007a; Blackburn *et al*, 2023).

The Nordic countries share the responsibility to secure their AnGR to the best of their knowledge (UN, 1992). Despite the increased efforts for utilizing cryoconservation as an additional safeguard, most of the Nordic breeds are not secured and, in some instances, important information regarding both the samples and donors is missing. The NordFrost project highlighted the benefits of increased Nordic collaboration on cryoconservation of AnGR. Nordic cooperation in the field of live animal conservation and cryoconservation benefits from the growing recognition of the value of genetic diversity, the identification of shared threats and opportunities, and the development of new cost-effective genebanking methods.

Stronger Nordic collaboration on cryoconservation of AnGR could improve both the risk status and genetic

diversity of the Nordic AnGR. Collaborative conservation could play an important role in strengthening the future of AnGR by allowing shared responsibility, cross-border cooperation and knowledge surrounding both infrastructure and data collection.

Supplemental data

Supplemental Material 1. National Strategies of the Nordic Countries
Supplemental Material 2. Fibroblast cell lines and advancing technologies

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Author contributions

ELFW – concept development, writing: original draft preparation, review and editing, data collection and analysis, visualization; MK – concept development, writing: review and editing, data curating, visualization; JP – concept development, writing: original draft preparation, review and editing; LM – concept development, writing: original draft preparation, review and editing; JK and PC– writing: review and editing; LLS, MK, TM and IM – concept development, writing: review and editing; MH – concept development, writing: original draft preparation, review and editing.

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Phenotypic characterization of cattle breeds in Southern Ethiopia: Implications for breed differentiation and conservation

Bergene Banjaw[a], **Habtamu Lemma Didanna**[a] **and Amine Mustefa** *[,b]

[a] *College of Agriculture, Wolaita Sodo University, Wolaita, Ethiopia*
[b] *Ethiopian Biodiversity Institute, Addis Ababa, Ethiopia*

Abstract: This study aimed to characterize and quantify the phenotypic relationship between Gamo and Gofa cattle breeds using nine morphometric measurements and 11 morphological traits. A total of 600 adult cattle (486 females and 114 males) were randomly selected from six purposively chosen districts. Univariate and multivariate analyses were conducted using Statistical Analysis Software. The univariate analysis revealed the morphometric values and morphological characteristics of both cattle breeds but did not show significant variations between them. The majority of the cattle exhibited uniformly patterned coat colour, upward-oriented, straight-shaped horns with black colour, laterally oriented ears with rounded edges, straight face profiles, small hump sizes, short coat hair, and medium tail length. In accordance with the phenotypic similarities observed in the univariate analysis, multivariate analysis also failed to identify significant differences between the two breeds. These results suggest that the two cattle breeds are phenotypically inseparable. However, these phenotypic similarities do not necessarily indicate genetic similarities. Therefore, further genetic characterization is recommended to assess the degree of genetic relationship between the breeds. In the meantime, it is advised to design breed-specific *in situ* conservation and genetic improvement programmes without separating the cattle breeds.

Keywords: Gamo-Gofa, morphological traits, phenotypic characterization, univariate analysis

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Introduction

Ethiopian indigenous cattle are vital to the livelihoods of smallholder farmers and significantly contribute to the nation's economy, particularly through their role in the agricultural GDP (CSA, 2022). These cattle are primarily valued for milk, meat and draught power, while also serving as sources of income, manure and cultural capital (Zerabruk and Vangen, 2005; Genzebu *et al*, 2012; Yimamu, 2014; Kebede *et al*, 2017; Getachew *et al*, 2020). With an estimated population of 70.3 million cattle, Ethiopia hosts the largest cattle herd in Africa (CSA, 2022; Statista, 2024), underscoring their prominence within the livestock sector.

Genetic diversity, both within and among breeds, is a critical foundation for conservation and genetic improvement strategies. Indigenous breeds dominate Ethiopia's cattle population, comprising 28 officially registered breeds (EBI, 2016; Mustefa, 2023). However, several gaps exist in breeds' documentation and characterization. For instance, several phenotypically studied breeds – including Bonga, Fellata, Gamo and Qocherie – remain unregistered, while others (e.g. Adwa, Hamer and Smada) lack comprehensive phenotypic data despite formal registration (Mustefa, 2023). Addressing these inconsistencies is essential to establish a nationwide framework for breed-specific conservation and genetic improvement programmes.

This study focused on two cattle breeds, Gamo and Gofa. The Gofa cattle breed, which was first studied by Rege and Tawa (1999), is officially registered in the Ethiopian indigenous cattle breeds database (EBI,

*Corresponding author: Amine Mustefa (aminemustefa32@gmail.com)

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2016). Gofa cattle, primarily used for work, milk and meat, were categorized as Small East African Zebu (SEAZ) (Rege, 1999). According to Rege and Tawa (1999), Gofa cattle are one of the smallest strains not only among the Abyssinian zebu but also among all Ethiopian cattle. Small hump, small to medium horns and dominantly red colour are some of their qualitative characteristics (Rege and Tawa, 1999). The study by Kebede *et al* (2017) on Gofa cattle has also reported the existence of diverse coat colours, patterns and qualitative traits. The name Gofa cattle was first introduced by Rege and Tawa (1999) and then by Kebede *et al* (2017) after the 'Gofa' ethnic community which raised them.

On the other hand, Gamo cattle, first studied by Chebo *et al* (2013), were not officially registered in the Ethiopian indigenous cattle breeds database (EBI, 2016). However, the study by Chebo *et al* (2013) showed the existence of potential cattle breeds in the area. According to Chebo *et al* (2013), the Gamo cattle were further divided into two subpopulations: the Gamo Highland and the Gamo Lowland. The Gamo Highland were relatively smaller with compact bodies compared to the medium-to-large-bodied Gamo lowland subpopulations. The name Gamo cattle was first introduced by Chebo *et al* (2013) after the 'Gamo' ethnic community which raised them.

The production system of both Gamo and Gofa cattle breeds was reported to be similar, with cattle owners practising comparable husbandry methods. Own and communal grazing lands were the source of feed, while natural and controlled breeding were the common breeding systems among the owners of both cattle breeds (Chebo *et al*, 2013; Kebede *et al*, 2017; Zeleke *et al*, 2017). However, as mentioned above, the two cattle breeds were studied separately and at different times. This hindered the comparison of the two breeds, which further affected the breed registration as well as the development of breed-specific breeding programmes. Therefore, an inclusive phenotypic characterization study was mandatory to understand the relationships between the breeds. Thus, the current study aimed to conduct an on-farm phenotypic characterization of Gamo and Gofa cattle, assess their morphological diversity, and quantify the degree of phenotypic divergence between them.

Materials and methods

Study areas

The study was carried out in the Gamo and the Gofa zones. Three districts were selected from each zone: Kucha, Daramalo and Dita districts from Gamo zone, as well as Zala, Denbagofa and Oyda districts from Gofa zone (Figure 1). Weather and agroecology-related information of the selected districts are presented in Table 1.

Site and animal selection

Representative samples of Gamo and Gofa cattle breeds were selected from their respective breeding areas. Information on their breeding regions and distribution zones was gathered using secondary sources. The Gamo cattle breed is reported to be native to the Gamo zone, with its distribution extending into the neighbouring Gofa zone (Rege and Tawa, 1999; Chebo *et al*, 2013). Thus, three districts – Kucha, Daramalo and Dita – were randomly chosen from Gamo zone to represent the indigenous Gamo cattle. Similarly, Gofa cattle are reported to be primarily found in Gofa zone, with their distribution reaching into the neighbouring Gamo zone (Rege and Tawa, 1999; Kebede *et al*, 2017). Accordingly, three districts – Zala, Denbagofa, and Oyda – were randomly selected from Gofa zone to represent the indigenous Gofa cattle. From each district, two sampling sites (known as 'Kebeles,' the smallest administrative units) were randomly chosen. Twenty-five households-raising cattle were then randomly selected from each sampling site. From each household, two unrelated adult cattle, aged four years and older, were randomly chosen. One male cattle was sampled every two households within each kebele. These animals were carefully monitored by their owners and trained labourers. Aggressive cattle that were unable to stand properly on flat ground were excluded from measurements.

Data collection

Morphometric and morphological data were collected following the FAO (2012) guidelines. Data collection was conducted in the morning to minimize the effects of feeding and watering on the measurements. Three researchers were involved in the data collection process: two handled the morphometric data, while the third recorded the morphological data. To minimize bias, the same researchers performed the data collection in all sites throughout the study. The animals were measured using a textile measuring tape in centimetres. A total of 600 cattle (486 females and 114 males) were subjected to nine morphometric measurements (Table 2) and 11 qualitative (morphological) traits (Figure 2, Table 3). For data analysis, the cattle were grouped into three age categories based on the classification by Tatum (2011): group one (3–5 years), group two (6–7 years) and group three (8 years and older).

Data analysis

The overall data analysis was carried out using the Statistical Analysis System (SAS) software 9.0 (SAS, 2002).

UNIVARIATE procedure for data normality test, the frequency procedure for morphological (qualitative) data analysis, and the general linear model (GLM) procedure for morphometric (quantitative) data analysis were used. Data analysis was carried out using the following model: $Y_{ijk} = \mu + X_i + Y_j + Z_k + e_{ijk}$ where

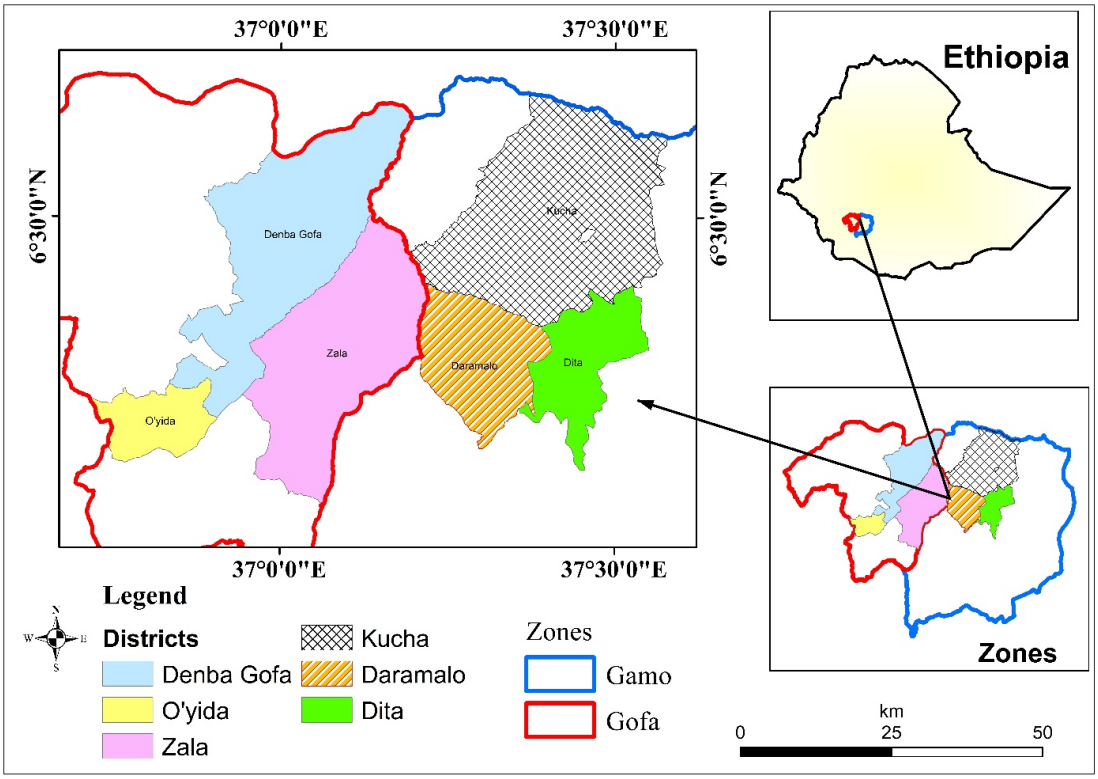

Figure 1. Map of the studied areas

Table 1. Weather and agroecology-related information of the selected districts (Gegnaw and Hadado, 2014; Leulalem *et al*, 2016; Kebede *et al*, 2017; Cholo *et al*, 2018; Chankalo, 2022; CSA, 2022; Kassa *et al*, 2022).

Parameters	Gamo zone			Gofa zone		
	Kucha	Daramalo	Dita	Zala	Dembagofa	Oyda
Human population	163,832	110,815	111,283	105,949	114,382	51,784
Cattle population	211,574	219,452	157,300	303,095	289,097	121,431
Temperature (°C)	20–25	19–22	10–23	18–32	18–28	15–25
Rain fall (mm)	1,100–1,600	1,300–1,900	2,500–3,500	500–900	900–1,100	1,000–2,000
Altitude	800–2,250	1,217–2,700	1,800–3,500	1,194–1,484	800– 2,860	1,000–3,200
Agroecology (%)						
Lowland	49.4	29.2	-	90	75	27
Midland	50.6	33.3	40	10	15	40
Highland	-	37.5	60	-	10	33

Table 2. Listof morphometric traits with their respective definitions. Measurements were conducted in centimetres (FAO, 2012).

No.	Morphometric traits	Definitions
1	Body length	Distance from shoulder point to pin bone
2	Heart girth	Chest circumference right behind its front two 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

Y_{ijk} is an observation, μ is the overall mean, X_i is the fixed effect of breed (i = Gamo, Gofa), Y_j is the fixed effect of sex (j = male, female), Z_k is the fixed effect of age (k = 3–5, 6–7, ≥8 years) and $e_{ijk}$is the random error. However, the effect of age on all qualitative traits was found to be not significant; hence, it is omitted from the model. Additionally, the quantitative data were analyzed separately for each sex by fitting breed as a class variable. Means (LSM) were separated using the adjusted Tukey-Kramer (Tukey, 1953; Kramer, 1956).

Multivariate analysis was carried out separately for each sex using both the morphometric and morphological traits at the same time. Prior to the analysis, the morphological traits were coded using discrete values. Stepwise discriminant analysis to detect morphometric traits that could better classify the cattle breeds, discriminant analysis to allocate individuals to known breeds and assess possibilities of misclassifications, and canonical discriminant analysis to deliver maximal separations between breeds were used. 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

The coat colour of the two studied breeds and sexes is presented in Figure 2. The coat colour was not significantly affected by breed, but it was affected by their sex. The majority of the studied cattle possessed red and light red coat colour. Sex-wise results revealed a high proportion of red-coloured females, while males possessed a light-red coat colour.

The effect of breed and sex on the qualitative characteristics of Gamo and Gofa cattle breeds is presented in Table 3 along with the respective chi-square values and levels of significance. Relatively, breed affected more morphological traits than sex: three out of the ten morphological traits were affected significantly by the breed of the animals, while sex affected only one trait. Accordingly, the majority of the studied cattle populations possessed uniformly-patterned coat colour, upward-oriented straight-shaped horns with black colour, laterally-oriented round edge ears, straight face profile, small hump size, short coat hair size, and medium tail length.

Morphometric traits

Tables 4 and 5 present the least square means, standard errors and pairwise comparisons showing the effect of breed, sex and age on the morphometric traits of the studied cattle populations. Breed affected two out of the nine morphometric traits, with Gofa cattle exhibiting greater pelvic width and horn length measurements compared to Gamo cattle. However, the effect of breed on the morphometric measurements was found to be sex-dependent. Breed significantly affected pelvic width and horn length measurements of the females, with values higher in Gofa females. However, these measurements of the male cattle populations were not significantly affected by breed differences. On the other hand, the heart girth measurement of the males was significantly affected by breed, with Gamo males having higher measurements than their counterparts from Gofa. However, the heart girth measurement of the females was not significantly affected by breed.

Similarly, sex affected seven out of the nine morphometric traits, with males exhibiting larger measurements than females in most of the traits except for ear and horn length. Age affected five of the nine morphometric traits, with most measurements increasing as the animal grew older. Body length and heart girth measurements were found to be stable after the age of six years. However, muzzle circumference and horn length measurements of middle-aged animals were found to be the highest among the compared age groups.

Multivariate analysis

Stepwise discriminant analysis

Ten out of the 20 morphometric and morphological traits were used to discriminate the female cattle populations while six traits were used to discriminate the males. The three most important morphometric variables used in discriminating the cattle breeds were horn length, pelvic width and coat colour pattern among females, and horn shape and horn length among males (Table 6). The overall results show the existence of low partial R-Square and F-values.

Discriminant analysis

Results of the discriminant analysis showed moderate classification of individual animals into their corresponding breed (Table 7). The highest classification into their respective breed was observed in Gamo males, while the lowest classification was observed in Gofa females.

Canonical discriminant analysis

Canonical correlations and eigenvalues for both male and female cattle populations are shown in Table 8. In line with the low partial R-Square and F-value outputs in Table 6, the eigenvalues were also small enough to discriminate between the two cattle breeds in both sexes. However, relatively higher Eigenvalues were observed for males than females. Similarly, the canonical correlation, which was used to build the canonical variate 1 (Can 1) from the used traits, was also low. However, a relatively higher canonical correlation was observed for males than females.

Pairwise squared Mahalanobis distances between the breeds were calculated as 1.43 for females and

Table 3. Percentages of qualitative characteristics of cattle populations by sex and breed.

Qualitative traits		Cattle Breed				Sex			
		Gamo (300)	Gofa (300)	χ^2 value	P	Males (114)	Females (486)	χ^2 value	P
Coat colour pattern	Uniform	85.3	78.0	5.4	NS	82.5	81.5	0.27	NS
	Patchy	9.7	14.0			10.5	12.1		
	Spotted	5.0	8.0			7.0	6.4		
Horn shape	Straight	69.3	59.0	7.0	**	65.8	63.8	0.16	NS
	Curved	30.7	41.0			34.2	36.2		
Horn orientation	Lateral	8.7	14.7	7.3	NS	15.8	10.7	13.2	*
	Upward	69.7	68.7			63.2	70.6		
	Downward	10.0	7.7			14.0	7.6		
	Forward	10.0	8.3			4.4	10.3		
	Backward	1.6	0.6			2.6	0.8		
Horn colour	Black	59.3	59.3	5.2	NS	50.9	60.7	4.6	NS
	Brown	9.0	6.3			9.6	7.2		
	White	28.0	33.7			35.1	29.8		
	Black + white	3.7	1.7			4.4	2.3		
Ear shape	Round edged	93.0	94.7	0.72	NS	91.2	94.4	1.7	NS
	Straight edged	7.0	5.3			8.8	5.6		
Ear orientation	Erect	16.3	20.0	10.5	**	18.4	18.1	2.0	NS
	Lateral	78.7	68.7			70.2	74.5		
	Dropping	5.0	11.3			11.4	7.4		
Face profile	Straight	83.7	90.7	7.0	*	89.5	86.6	1.5	NS
	Concave	13.0	8.0			9.6	10.7		
	Convex	3.3	1.3			0.9	2.7		
Hump size	Absent	5.3	3.3	2.7	NS	7.9	3.5	0.27	NS
	Small	61.3	59.7			54.4	61.9		
	Medium	32.3	35.0			35.1	33.3		
	Large	1.1	2.0			2.6	1.3		
Coat hair length	Short	93.7	94.0	0.03	NS	93.9	93.8	1.6	NS
	Medium	5.3	5.0			6.1	5.0		
	Long	1.0	1.0			0	1.2		
Tail length	Short	4.3	6.0	0.86	NS	2.6	5.8	1.9	NS
	Medium	74.0	73.0			76.3	72.8		
	Long	21.7	21.0			21.1	21.4		

Table 4. The effect of breed on the cattle morphometric measurements by sex. Measurements are in centimetres. N, number of animals sampled; BL, Body length; HG, Heart girth; HW, Height at withers; PW, Pelvic width; MC, Muzzle circumference; EL, Ear length; HL, Horn length; CBL, Cannon bone length; HC, Hock circumference; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.0001$; NS, Not significant.

Traits	Aggregate sex			Females			Males		
	Gamo	Gofa	Sig.	Gamo	Gofa	Sig.	Gamo	Gofa	Sig.
N	300	300		249	237		51	63	
BL	109.1±0.37	109.4±0.35	NS	106.9±0.33	107.3±0.34	NS	111.4±0.87	111.1±0.81	NS
HG	137.3±0.40	137.1±0.39	NS	134.1±0.36	135.4±0.37	NS	142.9±0.86	136.0±0.80	***
HW	108.9±0.43	108.8±0.41	NS	106.2±0.38	106.2±0.39	NS	112.2±1.13	111.4±1.05	NS
PW	31.3±0.21	32.9±0.21	***	30.7±0.18	32.4±0.18	***	32.0±0.61	33.0±0.57	NS
MC	36.9±0.15	36.8±0.14	NS	36.4±0.14	36.3±0.14	NS	37.2±0.34	37.1±0.31	NS
EL	17.0±0.11	17.3±0.11	NS	17.3±0.10	17.7±0.11	NS	16.9±0.22	16.6±0.20	NS
HL	16.5±0.21	19.0±0.21	***	17.2±0.20	20.1±0.20	***	16.6±0.41	17.6±0.38	NS
CBL	25.8±0.15	25.7±0.14	NS	25.7±0.14	25.6±0.14	NS	25.7±0.31	25.8±0.29	NS
HC	30.4±0.17	30.6±0.17	NS	30.2±0.16	30.4±0.17	NS	30.8±0.37	30.9±0.35	NS

Table 5. The effect of sex and age on the cattle morphometric measurements. Measurements are in centimetres. N, number of animals sampled; BL, Body length; HG, Heart girth; HW, Height at withers; PW, Pelvic width; MC, Muzzle circumference; EL, Ear length; HL, Horn length; CBL, Cannon bone length; HC, Hock circumference; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.0001$; NS, Not significant.

Traits	Sex			Age			
	Males	Females	Sig.	< 6	6–7	> 7	Sig.
N	114	486		169	234	197	
BL	111.3±0.51	107.1±0.25	***	107.8 ± 0.44^{b}	109.7 ± 0.38^{a}	110.1 ± 0.45^{a}	**
HG	139.6±0.56	134.8±0.27	***	135.9 ± 0.48^{b}	137.8 ± 0.42^{a}	137.8 ± 0.50^{a}	**
HW	111.4±0.60	106.3±0.29	***	107.5 ± 0.51^{b}	108.7 ± 0.45^{b}	110.4 ± 0.53^{a}	***
PW	32.6±0.30	31.6±0.14	**	31.7±0.26	32.4±0.22	32.3±0.26	NS
MC	37.3±0.21	36.3±0.10	***	36.3 ± 0.17^{b}	37.4 ± 0.15^{a}	36.7 ± 0.18^{b}	***
EL	16.9±0.15	17.5±0.07	**	17.2±0.13	17.1±0.12	17.2±0.14	NS
HL	16.9±0.30	18.6±0.14	***	16.4 ± 0.26^{c}	19.0 ± 0.22^{a}	17.8 ± 0.26^{b}	***
CBL	25.9±0.20	25.7±0.10	NS	25.7±0.17	25.9±0.15	25.7±0.18	NS
HC	30.7±0.24	30.2±0.12	NS	30.5±0.21	30.4±0.18	30.5±0.21	NS

Table 6. Order of traits used in discriminating between the two cattle populations using a stepwise discriminant analysis (STEPDISC).

Sex	Step	Variables entered	Partial R-Square	F value	Pr > F	Wilks' Lambda	Pr < Lambda
Females	1	Horn length	0.1630	94.25	< 0.0001	0.8370	< 0.0001
	2	Pelvic width	0.0522	26.60	< 0.0001	0.7933	< 0.0001
	3	Coat colour pattern	0.0121	5.91	0.0154	0.7837	< 0.0001
	4	Canon bone length	0.0099	4.79	0.0291	0.7760	< 0.0001
	5	Face profile	0.0085	4.12	0.0428	0.7694	< 0.0001
	6	Body length	0.0096	4.63	0.0319	0.7620	< 0.0001
	7	Horn shape	0.0076	3.64	0.0570	0.7562	< 0.0001
	8	Muzzle circumference	0.0074	3.55	0.0600	0.7506	< 0.0001
	9	Hair length	0.0065	3.10	0.0792	0.7458	< 0.0001
	10	Horn orientation	0.0047	2.22	0.1368	0.7423	< 0.0001
Males	1	Heart girth	0.2461	35.25	< 0.0001	0.7539	< 0.0001
	2	Horn shape	0.1164	14.10	0.0003	0.6661	< 0.0001
	3	Horn length	0.0585	6.58	0.0117	0.6272	< 0.0001
	4	Hump size	0.0497	5.49	0.0210	0.5960	< 0.0001
	5	Pelvic width	0.0388	4.19	0.0431	0.5729	< 0.0001
	6	Hock circumference	0.0315	3.35	0.0701	0.5548	< 0.0001

Table 7. Number and (percentage) of observations classified into breed.

Sex	Breed	Gamo	Gofa	Total
Females	Gamo	**187 (75.10)**	62 (24.90)	249 (100)
	Gofa	80 (33.76)	**157 (66.24)**	237 (100)
	Error rate	0.2490	0.3376	**0.2933**
Males	Gamo	**43 (86.00)**	7 (14.00)	50 (100)
	Gofa	16 (26.67)	**44 (73.33)**	60 (100)
	Error rate	0.1400	0.2667	**0.2033**

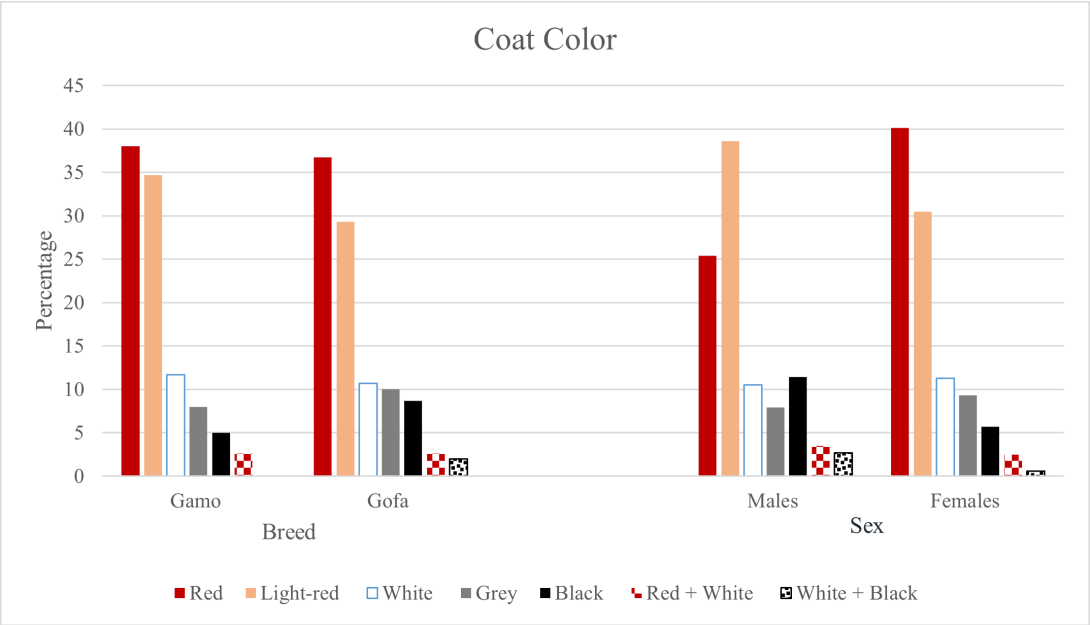

Figure 2. Effect of breed on coat colour (chi-square value 11.2, p = 0.0836), and effect of sex on coat colour (chi-square value 15.9, p = 0.0142).

3.68 for male cattle, indicating that males were more distantly related than females. However, both distances were small and insufficient to indicate a significant distance between the breeds. The overall multivariate analysis results showed low and non-significant distances between Gamo and Gofa cattle breeds.

Plots of the first two canonical variables to discriminate the cattle breeds are presented in Figure 3. In line with the result of the Mahalanobis distances, the studied Gamo and Gofa breeds were found to be inseparable and categorized in the same group, while a relative separation was observed between the males.

Discussion

Qualitative characteristics

Qualitative traits, due to their easily observable nature, are valuable for distinguishing between cattle breeds. Coat colour and coat colour patterns are among the most easily observed traits used to differentiate breeds. However, in this study, these traits did not distinguish the Gamo and Gofa cattle breeds, as most animals from both breeds exhibited uniformly patterned red and light red coat colours. The similarities in coat colour and pattern, along with other morphological and morphometric similarities between the breeds, may suggest genetic relatedness (Mustefa *et al* (2021) for Raya cattle; Getachew *et al* (2014) and Mustefa *et al* (2023) for Ogaden cattle). Therefore, the observed similarities in coat colour and pattern between Gamo and Gofa cattle imply phenotypic resemblance, which may reflect underlying genetic similarities. These findings, however, should be validated through genetic analysis.

The red-dominant coat colour observed in this study aligns with the findings of Rege and Tawa (1999), who identified red as the primary coat colour in Gofa cattle. Similarly, Kebede *et al* (2017) also reported that red and white were the most common coat colours in Gofa cattle. In agreement with these findings, Chebo *et al* (2013) reported that dark and light red were the predominant coat colours in Gamo cattle. The farmers' preference for red coat colour in both breeds may be linked to farmers' selection criteria, as red is a preferred colour in the studied areas. According to Kebede *et al* (2017), coat colour was a significant selection criterion for farmers, following milk yield.

Similarities in other qualitative traits, such as horn, ear, hump, face, hair, and tail lengths, were also observed between the Gamo and Gofa cattle breeds, which challenges their classification as distinct breeds. The minor differences observed could be attributed to variations within the breeds. Such intra-breed variations across different sampling locations were also reported by Terefe *et al* (2015) in Mursi cattle, Mustefa *et al* (2021) in Raya cattle, and Mustefa (2023) in Harar cattle.

Table 8. Multivariate statistics outputs of the canonical structures.

Multivariate Statistics	Females	Males
Canonical correlation	0.5140	0.7087
Eigen value	**0.3591**	**0.9060**

Morphometric traits

Morphometric measurements, in conjunction with qualitative traits, provide reliable information for assessing the degree of relationship between breeds. Most of the

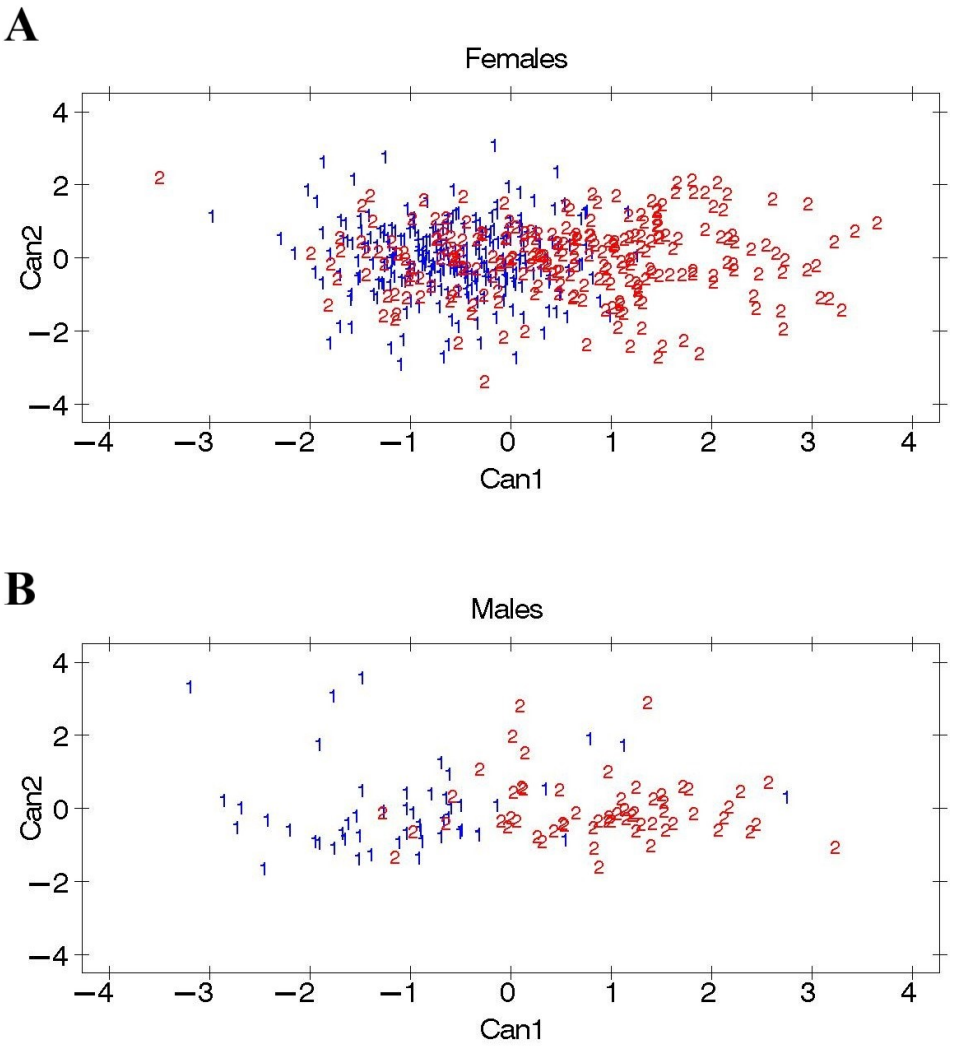

Figure 3. Plots of canonical discriminant analysis based on morphometric traits. A, females; B, males. Breed is indicated by numbers: 1, Gamo; 2, Gofa.

morphometric measurements were similar between the two cattle breeds, which supports the observed qualitative similarities. Consequently, no significant differences were found between the Gamo and Gofa cattle breeds that could serve to differentiate them. As mentioned in the qualitative section, the slight differences observed may reflect within-breed variations. These variations are crucial for designing conservation and genetic improvement programmes. Terefe *et al* (2015) in Mursi cattle, Mustefa *et al* (2021) in Raya cattle, and Mustefa (2023) in Harar cattle also reported morphometric variations within the same breed across different locations.

In comparison with the previous study on Gofa cattle by Kebede *et al* (2017), the Gofa females in this study showed similar body length, heart girth and wither height. However, they had larger muzzle and hock circumferences, and lower ear and horn lengths. In contrast, the Gofa males in this study exhibited comparable body length but lower values for other morphometric measurements. These findings suggest a reduction in body size of Gofa males over the past seven years, possibly due to negative selection practices by farmers. Similarly, most of the morphometric measurements for the cattle breeds in this study were lower than those reported by Chebo *et al* (2013) for Gamo cattle, but comparable to those reported by Zeleke *et al* (2017).

The Gamo and Gofa cattle breeds were found to be smaller than many other Ethiopian indigenous cattle breeds. This observation is consistent with Rege and Tawa (1999) description of Gofa cattle as the smallest strain of Ethiopian cattle. Their morphometric measurements were smaller than those of breeds such as Afar (Tadesse *et al*, 2008), Begait (Ftiwi, 2015), Begaria (Getachew *et al*, 2020), Fogera (Girma *et al*, 2016), Gojjam Highland (Getachew and Ayalew, 2014), Harar (Mustefa, 2023), Kereyu (Nigatu and Tadesse, 2020), Mursi (Terefe *et al*, 2015), Nuer (Minuye *et al*, 2018), Ogaden (Mustefa, 2023) and Raya cattle (Mustefa *et al*, 2021). However, their measurements were larger than those of Abergelle and Irob cattle breeds (Zegeye *et al*, 2021). Similar morphometric traits

were also observed in Arado (Genzebu *et al*, 2012) and Horro cattle (Bekele, 2015).

Multivariate analysis

High partial R-Square and F-values are necessary to demonstrate significant discrimination between populations. Additionally, low error rates are essential to show the distinctiveness of separate breeds. However, the results from the stepwise analysis (Table 6) exhibited low partial R-Square and F-values, indicating weak discriminatory potential of the morphometric traits. Furthermore, high error rates (Table 7) were observed, indicating greater similarities between the cattle populations, which lowers the likelihood of classifying the breeds into separate clusters. In contrast, lower error rates would indicate distinct differences between the breeds. For example, an error rate of 1% was reported in the classification of phenotypically unrelated Harar and Ogaden cattle breeds (Mustefa, 2023).

An eigenvalue greater than 1 is required for breed discrimination. If the value falls below 1, discrimination between the studied animals is not significant. The lowest eigenvalues observed in both sexes (Table 8) failed to distinguish the cattle populations into separate clusters, suggesting the absence of two distinct breeds. Similarly, high Mahalanobis distances between breeds are needed for clear cluster separation, but the Mahalanobis distance results in this study were low, with males showing slightly higher distances. This could be attributed to the smaller sample size of oxen. The accuracy of the analysis improves with larger sample sizes. Due to the low eigenvalue (< 1) and short Mahalanobis distances in the multivariate analysis, the studied cattle breeds were found to be phenotypically inseparable. However, phenotypic similarities do not necessarily imply genetic similarities between breeds (Zechner *et al*, 2001).

Effect of sex and age

Sex and age had minimal effects on the qualitative traits of the studied cattle populations since qualitative traits are typically controlled by fewer genes (Falconer, 1989). Therefore, successive planned selection activities are required to bring changes to the qualitative traits. On the other hand, the morphometric traits were influenced by both sex and age, because quantitative traits are influenced by a greater number of genes (Falconer, 1989). This means a few natural or artificial selection activities can produce significant changes to the quantitative traits.

Accordingly, males were generally larger than females in most morphometric traits of both breeds, aligning with Rensch's rule (Rensch, 1950), which suggests that females of a species are usually smaller than males. These differences could be attributed to testosterone, which promotes the development of skeletal and muscle mass in males (Baneh and Hafezian, 2009). The effect of the endocrine system on growth was also significant, with estrogen's influence on growth being more limited in females (Chriha and Ghadri, 2001; Baneh and Hafezian, 2009). Similar findings, showing male dominance in size, were reported by Mustefa (2023) for Harar and Ogaden cattle breeds, Mustefa *et al* (2021) for Raya cattle, and Terefe *et al* (2015) for Mursi cattle.

Age significantly affected five morphometric traits. Body length and heart girth measurements showed stable body development from the middle-aged group, while muzzle circumference and horn length measurements indicated the middle-aged group as optimal for these traits.

Significantly different breeds need to be registered separately, while the same breed should be registered only once. This is because our next step as a country is to design breeding programmes that include both conservation and genetic improvement activities for each breed individually. Therefore, conducting these programmes separately for breeds without significant differences would be inappropriate. The currently observed differences among these breeds can be considered as within-breed variation; however, this needs to be supported by further genetic characterization studies.

Conclusion

In accordance to the observed similarities in morphological and morphometric traits between Gamo and Gofa cattle breeds, multivariate analysis failed to identify significant differences, suggesting that the two breeds are inseparable. However, phenotypic similarities do not necessarily indicate genetic similarity. Therefore, further genetic characterization is recommended to assess the genetic relationship between these breeds. In the meantime, the studied cattle populations should not be regarded as separate breeds. Breed-specific *in situ* conservation and genetic improvement programmes should consider the cattle populations as a single entity. Additionally, a unified breed name that can represent both populations is recommended for consideration by the country's National Advisory Steering Committee for Animal Genetic Resources.

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Strategies to balance productivity and genetic diversity for the sustainable use of indigenous livestock breeds: A case study of Ethiopia

Amine Mustefa *

Ethiopian Biodiversity Institute, Comoros street, Addis Ababa, Ethiopia

Abstract: Livestock genetic improvement and conservation approaches follow divergent paths to achieve livestock productivity and genetic diversity, respectively. However, designing a win-win solution is mandatory to secure sustainable utilization of indigenous livestock breeds. To recommend a balanced solution, a systematic review was conducted to summarize the advantages and limitations of both approaches in developing countries using Ethiopia as a case study. Within-breed selection, breed substitution and crossbreeding programmes were implemented to achieve livestock genetic improvement while *in situ* and *ex situ* methods were used to maintain the genetic diversity of the indigenous livestock breeds. The genetic improvement approach offers advantages such as increased productivity, climate change mitigation and reduced animal aggression. However, it is also associated with limitations, including genetic erosion, maladaptation, inbreeding, high costs, and longer time requirements. On the other hand, the conservation approach focuses on maintaining genetic diversity, adaptable breeds, unique traits, cultural heritage and market-demanded products. However, maintaining indigenous breeds without genetic improvement is often associated with lower productivity, which hinders food security and income generation for farmers. Therefore, a balanced application of both approaches is recommended to achieve optimal productivity while preserving the genetic diversity of indigenous breeds. To ensure sustainable utilization, it is recommended to identify indigenous livestock breeds through phenotypic, genomic and historical characterization; conduct breed-, sex- and age-specific population censuses; evaluate breeds on station and on farm; delineate conservation areas; implement cryoconservation; and improve husbandry practices.

Keywords: Adaptability, climate change, conservation, genetic erosion, inbreeding

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Introduction

In many developing countries, livestock (cattle, sheep, goats, poultry, camels, horses and donkeys) play a vital role in rural economies (CSA, 2021). Livestock provide meat, milk, eggs and other products essential for human nutrition. Livestock also serve as a source of income, draught power and manure for crop production. Moreover, livestock is deeply intertwined with cultural practices and traditions, making it an integral part of the social fabric (Adane and Girma, 2008; Gizaw, 2009; CSA, 2022). The sector is dominated by indigenous animals that have evolved over centuries and are managed in diverse production environments, including lowlands, highlands, arid and semi-arid areas (EBI, 2016; Assefa and Hailu, 2018).

However, the sustainable use of livestock in most developing countries has been significantly affected by two major challenges: climate change and the poor productivity of indigenous breeds. Climate change is one of the most pressing challenges of our time, with far-reaching impacts on agriculture and food security (El-Bilali *et al*, 2020). Developing countries, which are often more vulnerable to climate variability, face significant risks to their livestock production systems. Climate change exacerbates existing challenges in livestock production, including water scarcity, feed

*Corresponding author: Amine Mustefa (amine.mustefa@ebi.gov.et)

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shortages and disease outbreaks (Degefu and Milkias, 2024). Rising temperatures and changing precipitation patterns can reduce the availability of pasture and water, leading to decreased productivity and increased mortality rates (Woldeyohannes *et al*, 2023). At the same time, the poor productivity of indigenous breeds poses a significant challenge to the livestock sector in many developing countries, limiting the sector's potential benefits. This issue stems from a combination of low genetic potential and environmental constraints, including inadequate feed, veterinary care and management practices (Gizaw, 2009; Mustefa, 2022).

Therefore, to ensure the sustainable use of livestock, addressing these two major challenges is essential. In this context, two primary strategies have emerged: genetic improvement and the conservation of indigenous breeds. Livestock production and productivity can be enhanced through the implementation of various genetic improvement programmes. Selective breeding, crossbreeding and breed substitution are viable options for improving the genetic potential of indigenous breeds (Philipsson *et al*, 2006). On the other hand, the application of *in situ* and *ex situ* conservation, or a combination of both can help maintain the diversity of indigenous livestock breeds, enabling them to cope with upcoming climate-driven changes. Indigenous livestock breeds are known for their ability to adapt, produce and reproduce under harsh environmental conditions, such as scarce feed and water, extreme temperatures, disease challenges and prolonged drought periods (EBI, 2016; Assefa and Hailu, 2018; Endris *et al*, 2022). In addition to their adaptability, indigenous breeds are valued for their desirable products, such as eggs, meat and milk. The market value of products from indigenous breeds is often higher than those from exotic breeds.

However, enhancing productivity and diversity simultaneously is challenging because the concepts of genetic improvement are often associated with decreasing diversity (EBI, 2016). Moreover, according to Article 2 of the Convention on Biological Diversity (CBD), sustainable use is defined as "the use of components of biological diversity in a way and at a rate that does not lead to the long-term decline of biological diversity, thereby maintaining its potential to meet the needs and aspirations of present and future generations" (CBD, 2004).

Therefore, balancing genetic improvement with the conservation of indigenous livestock breeds is crucial. To achieve this, it is necessary to review the principles and on-the-ground impacts of these approaches. Thus, the current study aims to recap the advantages and limitations of both options (conservation and genetic improvement) for the sustainable utilization of indigenous livestock breeds with a focus on cattle, sheep, goats and chicken, using Ethiopia as a case study, and to recommend a win-win solution.

A systematic review was conducted, following five steps as stated in Khan *et al* (2003):

- Step 1: Framing review questions. The review question focused on the advantages and limitations of livestock genetic improvement and conservation programmes related to the sustainable utilization of the livestock production sector.
- Step 2: Identification of relevant work. Relevant published articles as well as unpublished MSc and PhD thesis works addressing the framed review questions were extensively searched.
- Step 3: Quality assessment. Articles published in reputable journals were selected alongside the MSc and PhD thesis works.
- Step 4: Summarization of evidence. Information related to the genetic improvement programmes using within-breed selection, crossbreeding and breed substitution approaches, as well as *in situ* and *ex situ* conservation programmes, was compiled.
- Step 5: Interpretation of findings. The main findings from Step 4 were interpreted by comparing the achievements and limitations of genetic improvement and conservation approaches, as well as examining them against scientific justifications.

Genetic improvement

Livestock genetic improvement refers to the enhancement of the genotype of live animal breeding populations to increase their productivity, efficiency and resilience (Mueller and Van Eenennaam, 2022; Tesfa *et al*, 2024). The primary goals of livestock genetic improvement activities in most developing countries are to increase meat, milk and egg production, as well as improve feed efficiency. This can be achieved through selective breeding, crossbreeding, breed substitution and the use of advanced biotechnologies such as genomic selection and gene editing (Belew *et al*, 2016; Haile *et al*, 2020; Woldeyohannes *et al*, 2023).

Within-breed improvement involves selecting superior animals from the same population to serve as parents for the next generation while culling low-performing animals from the flock (Haile *et al*, 2020). Crossbreeding improves the genotype of indigenous animals by crossing them with high-performing exotic breeds. Breed substitution, on the other hand, involves replacing low-performing indigenous animals with high-performing exotic breeds (Vaccaro and Steane, 1990; Solomon *et al*, 2014).

Ethiopia has successfully implemented genetic improvement programmes of cattle (Beneberu *et al*, 2021), sheep (Getachew *et al*, 2020), goats (Solomon *et al*, 2014), and chicken (Yigzaw *et al*, 2024). Alongside within-breed selective breeding initiatives, numerous crossbreeding and breed substitution programmes have been implemented, introducing several exotic cattle, chicken and small ruminant breeds (Table 1).

Table 1. Exotic livestock breeds that were introduced into Ethiopia in the past decades

Livestock species/breeds	Introduction year	References
Cattle		
Angus	1950s	Tucho *et al* (2021)
Brahman	1950s	Chebo and Alemayehu (2012)
Brown Swiss	1947	Hunde (2018)
Hereford	1950s	Tucho *et al* (2021)
Holstein-Friesian	1950s	Albero (1983)
Jersey	1987	Beneberu *et al* (2021)
Simmental	1950s	Mwenya (1992)
Sheep		
Awassi	1980	Getachew *et al* (2020)
Corriedale	1967	Getachew *et al* (2016)
Dorper	2007	Habtegiorgis *et al* (2025)
Hampshire	1967	Sheriff and Alemayehu (2018)
Merino	1944	Getachew *et al* (2016)
Rambouillet	1967	Tibbo *et al* (2006)
Romney	1967	Sheriff and Alemayehu (2018)
Goats		
Anglo-Nubian	1970s	Workneh (2000)
Boer	2007	Mustefa *et al* (2019b)
Saanen	1940s	Awgichew *et al* (1989)
Toggenburg	1975	Girma *et al* (2000)
Chicken		
Arbor Acre	2000s	Alemneh and Getabalew (2019)
Australorp	1953	Gage and Suntebo (2023)
Bovans Brown	1950s	Melkamu *et al* (2017)
Brown Leghorn	1950s	Chebo *et al* (2022)
Cobb-500	2000s	Sidrak *et al* (2021)
Dominant Brown D102	2000s	Guteta (2021)
Dominant Sussex	2000s	Yigzaw *et al* (2024)
Fayoumi	1996	Geleta *et al* (2013)
Hubbard Classic	2015	Fekadu *et al* (2022)
Hubbard JV	2015	Tolasa (2021)
ISA Brown	1950s	EBI (2016)
Koekoek	1950s	Abadi *et al* (2020)
Lohman Brown	1950s	Kidie *et al* (2024)
Lohmann Silver	2022	Fekadu *et al* (2022)
New Hampshire	1953	Gage and Suntebo (2023)
Novo Brown	1950s	Yigzaw *et al* (2024)
Rhode Island Red (RIR)	1953	Hussen and Anja (2017)
SassoT44	2014	Chebo *et al* (2022)
Sussex	1950s	Chebo *et al* (2022)
White Leghorn	1953	Chebo *et al* (2022)

Advantages of livestock genetic improvement programmes

Increased Productivity

The primary significance of genetic improvement methods lies in enhancing productivity (Mueller and Van Eenennaam, 2022). The objectives of genetic improvement programmes can differ with focuses on aspects like growth, production and reproductive performance. Some of the reported results for each species are presented below.

Cattle

Most of the cattle genetic improvement programmes carried out in Ethiopia so far were aimed at increasing milk yield (Getahun *et al*, 2020). The lactation milk yield results of both the indigenous and crossbred cows are presented in Table 2. Notable differences were observed between the indigenous and crossbred cows where the latter performed more than threefold in most cases. The Holstein Friesian crosses were observed to produce better milk yield than the Jersey crosses. Furthermore, the on-farm results were lower than the on-station reports. This might be due to the suboptimal management practices of the farmers as well as limited adaptability of the crossbred animals to the local environment.

Small ruminants

Thus far, most of the small ruminant genetic improvement programmes carried out in Ethiopia have targeted growth traits (Tesema *et al*, 2020). Body weight results from birth to yearling age of both the indigenous and crossbreds are presented in Table 3. Differences were observed between the indigenous and crossbreds in most cases. Most of the crossbreds had better growth performances than the indigenous breeds except for Afar sheep and Central Highland Goat crosses where the indigenous performed better than the crosses. This might be due to the lower adaptability of the crossbreds which were managed on station with intensive and semi-intensive management systems. On the other hand, community-based breeding programmes (CBBP) were observed to bring outstanding results in Bonga sheep while their effect was small in Menz sheep and Abergelle goats.

Chicken

Chicken genetic improvement programmes primarily aimed to improve egg production, while growth and reproduction traits were also given due consideration (Dana *et al*, 2010; Esatu, 2015; Chebo *et al*, 2022). According to Alemneh and Getabalew (2019), the overall egg production of the Ethiopian indigenous chicken breeds was reported to be 30–60 per hen per year. Compared to this value, notable productivity gain were obtained through the implementation of genetic improvement programmes under intensive and extensive management systems (Table 4). Extremely lower egg production was also observed for Sasso (133) and Bovans Brown (124), which might be due to adaptation problems under certain production conditions (Assefa *et al*, 2019; Litigebew *et al*, 2021).

Similarly, notable successes have also been documented using the within-breed selection approach where a 21% egg number increment at 24 weeks was reported for the indigenous Horro chicken selective breeding programme (Esatu, 2015). Moreover, improved Horro chicken showed a 124% egg increment by week 45 (Wondmeneh *et al*, 2016), and were also reported to produce 150 eggs/hen/year, which is significantly higher than the egg production per year of the unimproved Horro chicken (Moges *et al*, 2010).

Climate change mitigation

Climate change, driven by rising temperatures, is among the factors limiting the sustainable use of animals and their products. The emission of greenhouse gases, including methane, carbon dioxide, nitrous oxide and halocarbons, is regarded as the primary driver of temperature increases. While livestock production is often seen as a victim of climate change, it is also identified as a major contributor to the process (Cassandro, 2020). Therefore, minimizing the contribution of livestock production to climate change is imperative. Genetic improvement is recognized as an important tool for mitigating climate change by reducing greenhouse gas emissions (Cassandro, 2020; Stranden *et al*, 2022). The intensification approach to genetic improvement, which reduces the total number of animals while improving their efficiency, has been reported to decrease emissions (Cassandro, 2020). According to Jardine *et al* (2012), reducing the number of animals could result in an estimated 8% drop in greenhouse gas emissions. Alongside decreasing animal numbers, increasing feed efficiency has been shown to significantly reduce greenhouse gas emissions in dairy production (Edwards-Jones *et al*, 2009; Bell *et al*, 2011). Hence, genetic improvement in addition to extensive pasture-based farming systems is regarded as a cost-effective approach to climate change mitigation (Cassandro, 2020; Stranden *et al*, 2022; Marchegiani *et al*, 2025).

Reducing aggressiveness

Animal temperament, or docility, is an important trait in cattle production, influencing not only human safety but also animal welfare and productivity (Norris *et al*, 2014). According to Norris *et al* (2014), poor animal temperament is associated with reduced performance, carcass quality and animal health. Thus, temperament affects the sustainable use of a given breed, with docile animals often preferred over aggressive ones (Dickson *et al*, 1969). Most indigenous breeds are reported to be more aggressive than exotic breeds. Therefore, crossing indigenous breeds with or replacing them with exotic breeds may reduce their aggressive temperament. One widely introduced cattle breed in most developing countries is the Holstein Friesian. According to Dickson *et al* (1969), Holstein Friesians, known for their high

Table 2. Lactation milk yield (kg) of representative indigenous and crossbred cows in Ethiopia. HF, Holstein-Friesian; JE, Jersey.

Breeds/Genotypes	Lactation milk yield	Breed type	Management	References
Arsi (AR)	809	Indigenous	On station	Niraj *et al* (2014)
50% HF x 50% AR	2,247	Crossbreds	On station	Million *et al* (2004)
75% HF x 25% AR	2,497	Crossbreds	On station	Million *et al* (2004)
50% JE x 50% AR	1,741	Crossbreds	On station	Niraj *et al* (2014)
Begait (BE)	672	Indigenous	On station	Tadesse and Dessie (2003)
50% HF x 50% BE	2,312	Crossbreds	On station	Tadesse and Dessie (2003)
75% HF x 25% BE	2,373	Crossbreds	On station	Tadesse and Dessie (2003)
50% HF x 50% BE	1,488	Crossbreds	On farm	Bekele *et al* (2011)
50% JE x 50% BE	970	Crossbreds	On farm	Bekele *et al* (2011)
Borana (BO)	771	Indigenous	On station	Demeke *et al* (2000)
50% HF x 50% BO	2,203	Crossbreds	On station	Getahun *et al* (2020)
75% HF x 25% BO	2,959	Crossbreds	On station	Getahun *et al* (2020)
50% JE x 50% BO	1,684	Crossbreds	On station	Gebregziabher *et al* (2014)
75% JE x 25% BO	1,832	Crossbreds	On station	Gebregziabher *et al* (2013)
Horro (HO)	559	Indigenous	On station	Gizaw *et al* (2011)
50% HF x 50% HO	1,836	Crossbreds	On station	Gebregziabher *et al* (2013)
75% HF x 25% HO	2,184	Crossbreds	On station	Gebregziabher *et al* (2013)
50% JE x 50% HO	1,621	Crossbreds	On station	Gebregziabher *et al* (2013)
75% JE x 25% HO	1,724	Crossbreds	On station	Gebregziabher *et al* (2013)

Table 3. Growth performance of some indigenous and crossbred small ruminants in Ethiopia. BHS, Black Head Somali; TU, Tumelie; DO, Dorper; AW, Awassi; CHG, Central Highland Goats; WG, Woyto Guji; BW, birth weight; WW, weaning weight; SMW, six months' weight; YW, yearling weight; IN, indigenous; CR, crossbred; OS, on station; OF, on farm; CBBP, community-based breeding programme.

Species Breed/Genotype	BW	WW	SMW	YW	Breed type	Management	References
Sheep							
Afar (AF)	2.7	11.5	-	26.6	IN	OS	Yibrah (2008)
50% DO x 50% AF	2.6	9.5	13.2	25.0	CR	OS	Abebe *et al* (2016)
BHS	2.5	11.3	-	23.1	IN	OS	Yibrah (2008)
50% DO x 50% BHS	3.0	15.1	-	-	CR	OS	Teklebrhan *et al* (2014)
Menz (ME)	2.1	9.1	-	17.3	IN	OS	Markos (2006)
50% DO x 50% ME	2.8	12.3	17.3	31.3	CR	OS	Abebe *et al* (2016)
Menz	2.6	9.0	13.3	19.9	IN	CBBP	Abebe *et al* (2020)
Tumele (TU)	2.4	8.5	11.9	22.4	IN	OS	Lakew *et al* (2014b)
50% DO x 50% TU	3.2	15.0	20.4	31.4	CR	OS	Lakew *et al* (2014b)
Wollo (WO)	1.9	10.8	15.7	21.6	IN	OF	Amare *et al* (2018)
50% AW x 50% WO	2.4	13.8	22.7	30.4	CR	OF	Amare *et al* (2018)
Bonga	-	-	16.7	-	IN	OF	MoA (2018)
Bonga	3.9	16.3	27.8	-	IN	CBBP	Arega *et al* (2024)
Goats							
Abergelle (AB)	2.2	6.9	9.5	14.2	IN	OF	Hagos *et al* (2018)
50% BO x 50% AB	2.9	15.3	19.6	27.9	CR	OS	Belay *et al* (2014)
Abergelle (AB)	2.0	7.2	10.1	15.9	IN	CBBP	Gobeze *et al* (2017)
CHG	2.0	9.0	13.8	20.6	IN	OF	Deribe and Taye (2013)
50% BO x 50% CHG	2.6	8.8	11.2	16.7	CR	OS	Mustefa *et al* (2019b)
WG	2.0	9.0	11.5	-	IN	OF	Zergaw *et al* (2016)
50% BO x 50% WG	2.8	11.6	16.2	29.2	CR	OS	Dea *et al* (2019)

Table 4. Egg production per hen per yearof exotic chicken breeds in Ethiopia under intensive and extensive production management.

Intensive management system		
Breed	**Eggs**	**References**
Bovans Brown	292	Melkamu *et al* (2017)
Lohman Brown	275	Kidie *et al* (2024)
Faoumi	160	Geleta *et al* (2013)

Extensive management system		
Breed	**Eggs**	**References**
Bovans Brown	218	Melkamu *et al* (2017)
Koekoek	176	Abadi *et al* (2020)
Sasso	133	Assefa *et al* (2019)
Bovans Brown	124	Litigebew *et al* (2021)

milk yield and good temperament, have been selected for docility over generations. Moreover, within-breed selection for more docile animals may reduce the aggressiveness of indigenous breeds; however, this type of selection is not commonly practised in developing countries, including Ethiopia. The Sheko cattle breed of Ethiopia, known for its trypano-tolerant ability, is also noted for its aggressiveness (Desta *et al*, 2011; Aleme and Mengistu, 2023). For this reason, farmers often choose to cross it with other relatively docile cattle breeds (Desta *et al*, 2011; Aleme and Mengistu, 2023). Therefore, reducing aggressiveness is another advantage of genetic improvement approaches.

Limitations of livestock genetic improvement programmes

Genetic erosion

The concept of genetic improvement in indigenous livestock breeds is often associated with a reduction in within- and among-breed genetic variation. Both the less destructive within-breed selection approach and the more destructive indiscriminate crossbreeding and breed substitution approaches contribute to decreasing genetic diversity in indigenous breeds (Belew *et al*, 2016; Woldeyohannes *et al*, 2023). Even though its effect is less severe than other methods, continued within-breed selection can lead to genetic erosion due to random genetic drift. This occurs when a few genes responsible for economically important traits are favoured, while a large proportion of genes responsible for survival and adaptation traits are lost. The more severe options, such as crossbreeding and breed substitution, are even more destructive, with their contribution to genetic erosion observable within a short period (Rahman *et al*, 2013). Genetic erosion, caused by genetic drift, reduces adaptive genetic variation, limiting evolutionary responses (Köhler-Rollefson and Mundy, 2010). Therefore, genetic erosion negatively affects the long-term sustainable utilization of indigenous livestock breeds. Thus, genetic improvement approaches contribute negatively to the future sustainable use of indigenous livestock breeds due to their role in the erosion of adaptive genotypes (Rahman *et al*, 2013).

Maladaptation

Maladaptation is a significant limitation of genetic improvement approaches using exotic livestock breeds. Crossbreeding and breed replacement are not always effective potentially due to the poor adaptation of exotic breeds to local environments (Köhler-Rollefson and Mundy, 2010). Morbidity and mortality rates of crossbred livestock breeds in different locations of Ethiopia are presented in Table 5. Accordingly, a significantly higher mortality rate was observed for the crossbreds of Boer goats with Central Highland and Woyto Guji goats indicating their suboptimal adaptability to local conditions. Similarly, higher morbidity and mortality rates were also observed for the crossbreds of Holstein Friesian cattle. Moreover, the lower egg production per hen per year presented in Table 4 for Sasso (133) and Bovans Brown (124) might be due to their maladaptation to the local environment (Assefa *et al*, 2019; Litigebew *et al*, 2021). Exotic breeds, developed and selected for specific environmental conditions, often perform poorly when introduced to new environments due to adaptation problems (Köhler-Rollefson and Mundy, 2010).

Inbreeding

Genetic improvement is associated with the selection and use of a few high-performing sires as parents for the next generation (Mueller and Van Eenennaam, 2022). The use of elite sires, increased selection pressure and reproductive technologies like artificial insemination (AI) increase the likelihood of offspring being half-siblings, leading to inbreeding in successive generations (De-Roos *et al*, 2011). One of the negative effects of inbreeding is inbreeding depression, where the increased likelihood of offspring inheriting two copies of harmful recessive genes leads to reduced fertility, vigour and overall fitness (Tongsiri *et al*, 2019; Lozada-Soto *et al*, 2021). Reduced genetic diversity is another negative effect of inbreeding. Inbreeding decreases genetic diversity within a population, making it more vulnerable to diseases, parasites and environmental changes. These factors decrease productivity and increase mortality rates, making livestock production less sustainable (Tongsiri *et al*, 2019; Lozada-Soto *et al*, 2021). Moreover, ethical concerns arise from inbreeding, as it can lead to increased suffering and reduced welfare for animals due to genetic defects and health problems (Frankham, 2005; Skotarczak *et al*, 2020). On the other hand, selecting traits that enhance animal welfare, such as reduced aggression, can lead to more humane and sustainable use of animal populations.

Cost and time

Implementing genetic improvement programmes can be expensive, requiring investments in infrastructure,

Table 5. Morbidity and mortality rates of crossbred livestock breeds in different locations of Ethiopia. Exotic breeds (HF, Holstein-Friesian cattle; DO, Dorper sheep; BO, Boer goats; Bovans Brown; Sasso); Indigenous breeds (GHC, Gojjam Highland Cattle; AM, Ambo cattle; GO, Gofa cattle; TU, Tumelie sheep; WL, Wolaita sheep; AD, Adilo sheep; CHG, Central Highland Goats); OS, on station; OF, on farm.

Breed/Genotype	Morbidity (%)	Mortality (%)	Management	Location	References
Cattle					
50% HF x 50% GHC	56.5	28.1	OF	Bahir Dar zuria	Ferede *et al* (2014)
50% HF x 50% GHC	65.0	37.0	OF	Gozamen	Ferede *et al* (2014)
50% HF x 50% AM	62	22.0	OF	Ada'a Liben	Wudu *et al* (2008)
50% HF x 50% GO	66.7	20.0	OF	Wolaita soddo	Assefa and Ashenafi (2016)
50% HF x 50% GHC	47.3	17.9	OF	Bahir Dar	Yeshwas (2015)
Sheep					
50% DO x 50 TU	-	7.0	OS	Tumelie	Lakew *et al* (2014a)
50% DO x 50 WL	-	28.4	OF	Mente Dubo	Habtegiorgis *et al* (2025)
50% DO x 50 AD	-	9.8	OS	Boloso	Gemiyo *et al* (2017)
Goats					
50% BO x 50 CHG	-	56.1	OS	Ataye	Mustefa *et al* (2019a)
75% BO x 25 CHG	-	64.0	OS	Ataye	Mustefa *et al* (2019a)
50% BO x 50 WG	-	48.0	OS	Jinka	Molla (2016)
50% BO x 50 WG	-	41.0	OS	Konso	Dea *et al* (2019)
Chicken					
Bovans Brown	-	3.2	OS	Mekelle	Melkamu *et al* (2017)
Bovans Brown	-	20.3	OF	Mekelle	Melkamu *et al* (2017)
Sasso	16.1	12.7	OS	Sidama	Hailegebreal *et al* (2022)

technology and skilled personnel (Wojtkowski, 2008; Biscarini *et al*, 2015). Moreover, genetically improved animals often require better management because the genetic modifications that enhance certain productivity traits can also create new vulnerabilities or amplify existing issues, making them more susceptible to environmental stressors and requiring more precise care to maintain their optimal health and productivity (Wojtkowski, 2008; Biscarini *et al*, 2015). However, smallholder farmers in developing countries often have limited access to the technology and resources needed to implement effective genetic improvement programmes. Therefore, the high cost of genetic improvement programmes can be a significant barrier for smallholder farmers in developing countries. In addition to higher costs, genetic improvement approaches also require considerable time. Genetic improvement is a long-term process, often taking many generations to achieve significant results. This can be a challenge for farmers who need immediate solutions to improve their livelihoods (Biscarini *et al*, 2015).

Conservation

Conservation of farm animal genetic resources refers to various human interventions aimed at maintaining the diversity of farm animal genetic resources, without genetic change as far as possible, to contribute to current and future food and agricultural needs (Henson, 1992). Conservation of indigenous breeds not only preserves their genotypes but also allows farmers and breeders to select and develop new breeds that can adapt and produce under changing environmental conditions, making this approach critically important for sustainable utilization (Gicquel *et al*, 2020).

Animals can be conserved using *in situ* and *ex situ* conservation methods. *In situ* conservation maintains live animal breeding populations in their production environments (Henson, 1992). Under this approach, the animals continue to contribute to the food and agriculture of their breeding areas. On the other hand, *ex situ* conservation maintains genetic resources outside their production systems. There are two ways of conserving genetic resources using the *ex situ* approach: *ex situ in vivo* and *ex situ in vitro*. *Ex situ in vivo* involves maintaining live animal breeding populations outside their production environments, while *ex situ in vitro* involves the cryopreservation of semen, oocytes, embryos, cells and/or tissues in genebanks (FAO, 2012a).

In recent decades, several *in situ* and *ex situ* conservation programmes have been implemented in Ethiopia (Table 6). The primary objective and progress of the *in situ* conservation programmes have been to create exotic-free breeding tracts for the mentioned breeds. Similarly, the *ex situ in vivo* approach conserves live animal populations at ranches or research centres to produce pure parental lines for genetic improvement programmes, but has been applied to two indigenous cattle breeds so far: the Sheko and Fogera cattle breeds (Tibbo *et al*, 2004). Moreover, the *ex situ in vitro* conservation programmes aim to preserve the semen of

the cattle breeds for future restoration purposes, but has been applied to five indigenous cattle breeds so far: the Sheko, Fogera, Borana, Begait, and Irob cattle breeds out of the country's registered 28 cattle breeds (Assefa *et al*, 2021). The success of these programmes has been directly linked to the restoration of dwindling population sizes. Restoration of endangered breeds requires more budget and time than other conservation programmes, which are typically carried out through successive awareness-raising campaigns. Below are some of the advantages of conservation approaches for the sustainable utilization of indigenous livestock genetic resources.

Advantages of conservation

Genetic diversity

One of the main advantages of conservation programmes is the maintenance of genetic diversity (Köhler-Rollefson and Mundy, 2010; Gicquel *et al*, 2020). Maintaining genetic diversity is crucial because once genes are lost, they cannot be replaced except through cumulative selection or mutation (Smith, 1984). Maintaining within- and among-breed variability supports current and future research and development activities. It also enhances the effectiveness of within-breed selection-based genetic improvement programmes. Genetic improvement is more attainable in highly variable populations than in populations with low variability. Highly variable populations provide opportunities for the development of specialized breeds. The creation of synthetic breeds for specific purposes through crossbreeding requires the conservation of pure parent stocks. Maintaining the variability of indigenous breeds is also essential for their ability to adapt, produce and reproduce under future environmental changes (Silva *et al*, 2019; Gicquel *et al*, 2020). Moreover, ensuring a diverse genetic pool helps secure a more reliable and resilient food supply, which is particularly important in the face of climate change and other unpredictable challenges that can impact food production (Köhler-Rollefson and Mundy, 2010). According to Smith (1984), the conservation of indigenous breeds provides alternative breeding stock for future changes in market demands, husbandry practices and climate-driven environmental changes. Therefore, the conservation of indigenous breeds maintains genetic diversity, which in turn supports the sustainability of livestock production.

Adaptability

Adaptation to local environments can be defined in various ways, including disease resistance and tolerance to harsh conditions. Many indigenous breeds have natural resistance to local diseases and parasites (Köhler-Rollefson and Mundy, 2010). For example, the Sheko cattle breed is known for its trypano-tolerance (Desta *et al*, 2011; Aleme and Mengistu, 2023). Such genetic resilience is invaluable in most developing countries, where access to veterinary care and modern disease control methods is limited. On the other hand, indigenous breeds are often well-suited to challenging environments, such as arid and mountainous regions. They can thrive on limited resources and withstand harsh weather conditions, making them valuable assets for food security in marginal areas. The overall adaptability of indigenous breeds is due to their long-term evolution in specific local environments. In addition, in the face of future climate change, indigenous breeds often offer several advantages over exotic breeds (Silva *et al*, 2019). Thus, the conservation of indigenous breeds makes them more efficient and sustainable under current local climatic and management conditions, as well as future climate change (Köhler-Rollefson and Mundy, 2010; Gicquel *et al*, 2020).

Unique traits

The conservation of indigenous breeds is significantly associated with maintaining their unique traits. For example, the conservation of Sheko cattle in southwest Ethiopia is directly linked to preserving their trypano-tolerant ability (Desta *et al*, 2011; Aleme and Mengistu, 2023). Similarly, other special traits of indigenous breeds have been reported, such as the screw horns of Racka sheep in Hungary (Bodo, 1994) and the seaweed-eating sheep (Balasse *et al*, 2019). Moreover, indigenous breeds are known to produce items with special qualities, such as coloured wool, super-fine fibre, and tasty products like milk, meat and eggs (Köhler-Rollefson and Mundy, 2010). Therefore, for local communities that have adapted to these traits, the sustainable approach is to conserve them rather than crossbreed or replace them with high-yielding exotic breeds.

Cultural heritage

Indigenous breeds are deeply woven into the fabric of many cultures, playing significant roles in various aspects of life, from sustenance and livelihoods to social customs, traditions and religious practices (Smith, 1984; Köhler-Rollefson and Mundy, 2010; Marsoner *et al*, 2018). Indigenous breeds are often associated with specific cultural identities and traditions. They may be used in ceremonial events, festivals and traditional practices, symbolizing heritage and cultural continuity. Similarly, in many cultures, indigenous breeds are used as sacrificial offerings in religious ceremonies, symbolizing devotion and gratitude (Marsoner *et al*, 2018; Silva *et al*, 2019). Therefore, the conservation of indigenous breeds helps preserve cultural identity and history, which in turn supports the sustainable use of these breeds.

Market demand

In most developing countries, consumers highly prefer products from indigenous livestock breeds over those from exotic breeds (Sharif and Farooq, 2004; Silva *et al*, 2019). Several traditional beliefs and scientific reasons

Table 6. Ethiopian indigenous livestock breeds under conservation.

Livestock breeds	Conservation type	References
Cattle		
Sheko	*In situ* & *ex situ in vivo*	Aleme and Mengistu (2023)
	Ex situ in vitro	Assefa *et al* (2021)
Fogera	*In situ* & *ex situ in vivo*	Tesfa *et al* (2024)
	Ex situ in vitro	Assefa *et al* (2021)
Borana	*In situ*	Tessema *et al* (2022)
	Ex situ in vitro	Assefa *et al* (2021)
Begait	*Ex situ in vivo*	Mekuriaw and Kebede (2015)
	Ex situ in vitro	Assefa *et al* (2021)
Begaria	*In situ*	Aseged *et al* (2023)
Raya	*In situ*	Assefa *et al* (2021)
Irob	*Ex situ in vitro*	Assefa *et al* (2021)
Sheep		
Washera	*In situ*	Amane *et al* (2010)
Menz	*In situ* & ex *situ in vivo*	Gizaw *et al* (2013)
Bonga	*In situ* & *ex situ in vivo*	Mustefa (2023)
Wollo	*In situ*	Assefa *et al* (2021)
Horro	*In situ* & *ex situ in vivo*	Molla (2020)
Gedeo	*In situ*	Assefa *et al* (2021)
Goats		
Highland	*In situ*	Assefa *et al* (2021)
Arsi-Bale	*In situ*	Assefa *et al* (2021)
Chicken		
Horro	*In situ*	Taye (2024)
Metekel	*In situ*	Assefa *et al* (2021)
Jarso	*In situ*	Assefa *et al* (2021)
Kundudo	*Ex situ in vivo*	Sufiyan (2022)

can explain this preference. In Sri Lanka, for example, it is traditionally believed that milk from indigenous cows has medicinal and therapeutic properties due to its low likelihood of causing milk allergies in humans (Rajapakshe *et al*, 2015). According to Silva *et al* (2019), milk from indigenous cows is preferred in the Southern Province of Sri Lanka due to its high-fat content, which produces a firm curd structure and good flavour. Scientifically, meat from indigenous chickens has been reported to have better physicochemical and sensory parameters than meat from commercial broilers (Rajapaksha *et al*, 2014). Senarathne *et al* (2016) reported high mineral and fat contents in eggs from indigenous chickens. Physical and chemical analyses by Lordelo *et al* (2020) indicated higher quality in eggs from indigenous chicken breeds in Portugal compared to commercial breeds in many characteristics. Therefore, due to consumer preferences, eggs, meat and milk from indigenous breeds have become highly priced products in most developing countries (Silva *et al*, 2019). Thus, maintaining indigenous breeds helps ensure the availability of these preferred products in the market which also improves the income of farmers.

Limitations of conservation

Maintaining indigenous livestock breeds is significant for securing the sustainable utilization of these genetic resources; however, it comes with its own set of challenges. Below some of the limitations are presented.

Lower productivity

Compared to modern, high-yielding exotic breeds, indigenous cattle breeds often have lower milk yields, slower growth rates and lower feed conversion efficiency. For example, the average milk yield of Ethiopian indigenous cattle breeds (1.32–2.19 litres/cow/day) (Ayalew *et al*, 2018) and the average egg production of most indigenous chicken breeds (45–75 eggs/hen/year (Tolasa, 2021) and 30–60 eggs/hen/year (Alemneh and Getabalew, 2019)) are significantly lower than those of their exotic counterparts. Similarly, the slower growth rates of indigenous livestock breeds mean it takes longer for them to reach market weight, leading to increased feeding costs and delayed returns on investment for farmers. Moreover, the lower feed conversion efficiency of indigenous breeds means they require more feed to produce the same amount of meat or milk compared to modern breeds under uniform management and controlled envi-

ronment. This increases production costs and reduces profitability. These factors can result in lower profits for farmers, making indigenous breeds less attractive to those who need to maximize their outputs to remain profitable. Thus, solely maintaining indigenous breeds can affect sustainable food security and income generation goals, which can further influence their sustainable use.

Discussion

In Ethiopia, livestock genetic improvement programmes have been implemented through within-breed selection, crossbreeding and breed-substitution programmes. The within-breed selection programmes were mainly implemented in small ruminants and chicken through community-based breeding programmes (CBBPs) and on-station selection programmes. Similarly, several exotic breeds of cattle, sheep, goats and chicken were also imported to conduct crossbreeding and breed-substitution programmes. Accordingly, notable achievements were reported in cattle milk yield, chicken egg production and growth performances of small ruminants. Alongside increasing livestock productivity, livestock genetic improvement programmes were also reported to minimize the aggression of indigenous livestock breeds. These programmes were also reported to contribute to climate change mitigation. However, despite these achievements, livestock genetic improvement programmes were reported to have some limitations. These include the facilitation of genetic erosion and inbreeding, maladaptation of the exotic and crossbred to the local production environment, the need for a long implementation time, and high costs for both importing and managing the high-producing exotic breeds. Similarly, conservation programmes were also reported to have advantages and limitations regarding sustainable utilization of the livestock production sector. The advantages of conservation programmes include the preservation of genetic diversity of indigenous adaptable breeds, the maintenance of unique traits and cultural heritage, and the availability of products from indigenous breeds that meet market demands. However, maintaining indigenous breeds without genetic improvement is often associated with keeping low-productivity animals, which hinders food security and income generation for farmers. Therefore, designing a balanced approach is recommended to achieve optimal productivity while preserving the genetic diversity of indigenous breeds.

The way forward

Although genetic improvement and conservation approaches are inherently opposite and cannot be applied simultaneously to the same livestock population, it is essential to find a win-win solution for the sustainable utilization of indigenous livestock genetic resources to optimize outcomes in the livestock production sector. To achieve this, some recommendations are proposed below.

Identification of indigenous breeds

Characterization of indigenous cattle breeds is a foundational step prior to any breeding programme (FAO, 2012b). Several variables need to be considered at this stage, including the assessment of morphometric and morphological traits, identification of their production environments (origin/breeding tract and distribution areas), identification of unique traits, cultural values, adaptability to harsh environments (e.g. extreme weather conditions and climate change), adaptability to limited resources (e.g. feed, water and veterinary care), and assessment of indigenous knowledge associated with these breeds (FAO, 2012b). Alongside phenotypic and environmental variables, assessing within-breed genetic diversity is necessary to identify a breed. Similarly, assessing the degree of genetic relationship with other indigenous breeds (population structure) is required to determine the number of breeds in the country. Therefore, phenotypic, genomic and historic characterization is recommended.

Breed-level population size census

After identification, conducting a breed-level population size census is essential to assess the endangered status of each breed. Therefore, data on the number of animals by breed, sex and age are required to determine whether conservation or genetic improvement programmes should be implemented. Conservation programmes can be recommended for breeds with small populations to help maintain their genotype. Based on their current population size, appropriate conservation methods can be selected. *In situ* and *ex situ* conservation methods can be applied to critically endangered breeds. An indigenous breed with a large population size and a wide distribution area may be considered for a controlled crossbreeding programme to enhance targeted traits.

Breed evaluation

The assessment of on-station and on-farm phenotypic performances – such as growth, production, reproduction and survival traits – is necessary to understand the potential of each breed. A genomic evaluation of a breed for specific traits is also essential to assess its genetic potential. This evaluation is crucial for selecting a breed, trait, method and location for genetic improvement. Furthermore, within-breed selection-based genetic improvement programmes are recommended for populations with high genetic diversity. In contrast, populations with low genetic diversity may require a controlled crossbreeding programme. Before implementing any livestock breeding programme, evaluating the complementarity of each parent breed is essential. Therefore, breed evaluation is mandatory.

Breed and area delineation for breeding programmes

The results of characterization, breed-level census and breed evaluation activities need to be used to identify suitable breeding programmes for each livestock breed and production environment. Accordingly, breeds and areas can be delineated either for conservation or genetic improvement programmes. Based on this, a conservation programme can be applied to the economically important and endangered livestock breeds. It is also advisable not to implement crossbreeding and breed-substitution programmes in the origin and breeding tract of the indigenous livestock breeds. Within-breed selection approaches can be considered in these areas to bring the desired genetic improvement. Areas out of the indigenous livestock breed origin can be considered for either crossbreeding or breed-substitution programmes based on the complementarity of these livestock breeds with exotic ones. Additionally, exotic livestock breeds can be recommended in commercial farms with intensive management systems and controlled environments.

In situ and *ex situ* conservation

In situ and *ex situ* conservation options can be applied simultaneously or separately for economically important endangered indigenous breeds according to the situation (FAO, 2012a). *In situ* conservation, the maintenance of livestock breeds in their natural production environment, can be carried out for livestock breeds with relatively higher population sizes. Establishing a community and designing an incentive-based approach can be considered during the *in situ* conservation programmes. Similarly, *ex situ* conservation can be applied to livestock breeds with alarming population size or as a complementary method to *in situ* conservation. *In vivo* and/or *in vitro* can be considered simultaneously or separately to the livestock breeds with dwindling population size. *Ex situ in vitro*/cryoconservation is an advanced method of preserving genetic material at extremely low temperatures, typically using liquid nitrogen (-196°C). This technique is widely used in the conservation of livestock breeds, wildlife, plant species and in human medicine (e.g. preserving sperm, eggs and embryos). For indigenous livestock breeds, cryoconservation is a powerful tool to protect genetic diversity and ensure the survival of rare or endangered breeds. The implementation of these methods is expensive and needs a more skilled workforce than the *in situ* method FAO (2012a).

Husbandry practices

Alongside genetic improvement, improving husbandry practices enhances livestock productivity and diversity by optimizing animal health, nutrition, breeding selection and environmental management (Dristan, 2025). These improvements lead to increased yields of meat, milk and eggs, and the development of better genetic traits, while also promoting a broader range of livestock breeds suited to various ecological conditions and market demands (Dristan, 2025). Therefore, improving husbandry practices is essential to ensure the sustainable use of indigenous livestock genetic resources.

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Conflicts of interest

The author declares that there are no conflicts of interest.

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Phenotypic traits in natural perennial ryegrass populations and relations to climate conditions at sites of origins across Europe

Silvia Bachmann-Pfabe [*,a,b,‡], **Mareike Kavka** [a,‡], **Anna Roschanski** [a,c], **Klaus J Dehmer** [a], **Jean-Paul Sampoux** [d] **and Evelin Willner** [a]

[a] *Satellite Collections North, Oil and Fodder Crop Collections, Genebank Department, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Inselstraße 9, 23999 Malchow (Poel), Germany*

[b] *Present address: Faculty of Agriculture and Food Sciences, Plant Nutrition and Soil Science, University of Applied Sciences Neubrandenburg, Brodaer Strasse 2, 17033 Neubrandenburg, Germany*

[c] *Present address: Institute of Botany and Landscape Ecology, University of Greifswald, Soldmannstraße 15, 17487 Greifswald, Germany*

[d] *National Research Institute for Agriculture, Food and the Environment (INRAE), Centre Nouvelle-Aquitaine-Poitiers, RD150, 8660, Lusignan, France*

Abstract: Perennial ryegrass (*Lolium perenne* L.) is one of the most important forage grass species in temperate climates. However, natural perennial ryegrass populations have been exploited to only a limited extent by breeders. Therefore, 41 ecotypic *Lolium perenne* populations collected across Europe were studied for their agronomic performance in a 3-year common garden experiment located in north-eastern Germany (Poel Island, Mecklenburg Western Pomerania). Agronomic performances were evaluated on 30 plants per population for 11 traits related to forage value and environmental adaptation. Population means for studied traits were correlated to the values of climate variables at their collection sites. Populations clearly differed in their phenotypic performance, and eight populations originating from Belgium, France and Germany outperformed the other populations by showing the lowest winter damage, strongest spring growth and regrowth capacity after cuts and low disease susceptibility. Specifically, in the first experimental year, trait performances, in particular winter damage, spring growth and heading date, were related to the local climate at the site of origin of populations. Acclimation to the climate conditions at the experimental site might explain why these correlations were less pronounced in the second and third experimental years. The characterized populations might now be considered to improve specific traits in breeding.

Keywords: ecotypes, phenotypic diversity, climate norms, *Lolium perenne*

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Introduction

Perennial ryegrass (*Lolium perenne* L.) is one of the most important grass species for forage and turf uses in temperate climates worldwide and has undergone intensive breeding efforts (Humphreys *et al*, 2010; Sampoux *et al*, 2011). Natural perennial ryegrass populations are an important source of diversity for forage and turf breeding goals, but their wide genomic variability has been only partially exploited by breeding activities (Blanco-Pastor *et al*, 2019). The adaptation to local environments results in the differentiation of local ecotypes of perennial ryegrass with potentially valuable variability to improve growth seasonality, disease resistance or winter hardiness (Humphreys,

*Corresponding author: Silvia Bachmann-Pfabe (pfabe@hs-nb.de)

‡both authors contributed equally to the manuscript

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1989; Charmet and Balfourier, 1991; Oliveira-Prendes *et al*, 1997; Hulke *et al*, 2007, 2008; Kemesyte *et al*, 2010; Willner *et al*, 2010; Bachmann-Pfabe *et al*, 2018). Breeding increased the cumulative dry matter yield of forage-type perennial ryegrass cultivars between 0.3 and 0.9% annually during the past 40 to 50 years (Wilkins and Humphrey, 2003; McDonagh *et al*, 2016). More specifically, summer and autumn yields increased, while spring dry matter yield remained almost unchanged (Sampoux *et al*, 2011). However, early spring growth is an important breeding target in order to extend the growing season of perennial ryegrass, to provide enough fodder in temperate grasslands early in the year (Goslee *et al*, 2017) and to adapt the growing period to a longer heat and drought summer season caused by ongoing climate change. For forage and grazing usage, plant breeders developed forage-type ryegrass cultivars with different maturity groups, from early heading types with high early-season yields to late heading types. The latter are best adapted to a long grazing season and permanent pasture use due to their increased summer and autumn growth (Humphreys *et al*, 2010; Laidlaw, 2005). Disease resistance, especially to crown rust, improved in registered ryegrass cultivars for forage as well as for turf usage (Sampoux *et al*, 2011, 2013). Other current breeding goals are increased winter hardiness and persistence (Kemesyte *et al*, 2010; Goslee *et al*, 2017), reduced aftermath heading (Hurley *et al*, 2007; McGrath *et al*, 2010), tolerance to heat and drought stresses as well as increased nutrient and water uptake efficiency (Crush *et al*, 2010; Bothe *et al*, 2018).

Because intensive breeding in perennial ryegrass is a rather recent effort (20^{th} century), useful traits of wild populations can still be easily integrated into breeding programmes (Blackmore *et al*, 2016; Blanco-Pastor *et al*, 2019, 2021). Documenting the relationship between the phenotype, including agronomic performance, of natural populations and the climatic conditions at their sites of origin, contributes to understanding climate adaptation in the natural diversity of perennial ryegrass. This is essential for future breeding progress, as it helps to identify desirable breeding goals and to point out suitable genetic resources to choose in genebanks or to collect *in situ*.

The aim of this study was to combine phenotypic and environmental information in order to explore the trait variation between natural *L. perenne* populations along a geographical and climatic range. Therefore, we cultivated 41 different natural *L. perenne* populations represented by 30 individuals per population in a spaced plant nursery and evaluated their agronomic performances over three years.

Material and methods

Collection sites and site-specific characteristics

In 2015, 41 natural populations of perennial ryegrass (*Lolium perenne* L.) were sampled across Europe. In each population, a small number of living tillers were collected from 30–35 individual plants. The collection sites spanned areas of 100 to 290,000m^2, with most sites larger than 1,000m^2 (Supplemental Table 1). The 41 populations were collected in Belgium (BEL), Germany (DEU), Estonia (EST), France (FRA), Great Britain (GBR), Lithuania (LTU), The Netherlands (NLD), Poland (POL), Portugal (PRT) and Serbia (SRB) (Figure 1).

The majority of the collection sites had an elevation ranging from 0 to 800m above sea level (masl). Populations from sites above 800masl originated from Germany (DEU1, 1,040m), Serbia (SRB1, 1,076m; SRB2, 1,159m) and France (FRA5, 1,200m). The altitude of the sampling sites as well as their climatic conditions (Blanco-Pastor *et al*, 2021) are displayed in Table 1. The annual mean temperature (bio1) of the collection sites ranged from 5.3°C (population GBR8) to 14.8°C (population FRA3). The annual precipitation (bio12) averaged 902mm over collection sites and ranged from less than 580mm for populations DEU7, DEU9, DEU10, EST1 and FRA3, to more than 1,300mm for populations DEU1, FRA1, FRA5 and GBR6. The average daily maximum temperature of the warmest 14-day period of the year (bio5) was the highest for populations FRA3, PRT1, PRT2 and SRB3 (more than 28°C). The average daily minimum temperature of the coldest 14-day period of the year (bio6) was lowest for LTU1, LTU2, SRB1 and SRB2 (less than -5°C). Furthermore, populations EST1, FRA3 and PRT2 originated from the driest sites (precipitation <85mm in the driest quarter (bio17), Table 1).

As reported in the biological status of accessions (FAO/Bioversity, 2015) and the accession source (COLLSRC), the populations were predominantly collected from wild and semi-natural habitats (SAMPSTAT = 100, 110, 120; see Supplemental Table 1). These comprised natural grasslands, semi-natural pastures where no sowing or ploughing took place during the previous 15 to 70 years or roadsides. After collection, living tillers of the populations were transferred to the German Federal Ex Situ Genebank of the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Satellite Collections North at Malchow/Poel, Germany for conservation. The passport data of the accessions are available via the IPK Genebank Information System GBIS (https://gbis.ipk-gatersleben.de) and are also provided in Supplemental Table 1. Individual plants within populations were pre-cultured in seedling trays using a turf-based planting substrate (Einheitserde Uetersen, Pikiererde, Uetersen, Germany) and then transferred to the field. The ploidy level on all plants grown afterwards in the experimental garden was confirmed as diploid (2x = 14) using flow cytometry at INRAE (UR P3F).

Table 1. Country of origin, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) accession number, elevation and bioclimatic norms (1989–2010) at the collection sites of 41 *L. perenne* populations sampled across Europe. Bioclimatic norms over the period 1989–2010 were computed as per Blanco-Pastor *et al* (2021) (see also Supplemental Table 2). bio1, annual mean temperature; bio5, average daily maximum temperature over the warmest 14-day period of the year; bio6, average daily minimum temperature over the coldest 14-day period of the year; bio12, cumulated annual precipitations; bio17, cumulated precipitations of the driest quarter of the year.

Country of origin	Accession no.	Altitude (masl)	bio1 (°C)	bio5 (°C)	bio6 (°C)	bio12 (mm)	bio17 (mm)
BEL1	GR 13048	216	9.9	24.5	0.04	871	178
BEL2	GR 13047	19	10.9	24.3	0.94	838	144
DEU1	GR 13042	1,040	5.9	21.8	-4.50	1,584	333
DEU2	GR 13041	748	8.0	23.1	-4.18	1,050	164
DEU3	GR 13043	338	9.3	24.5	-1.30	860	169
DEU4	GR 13030	33	10.1	25.4	-0.63	696	135
DEU5	GR 13031	0	9.7	23.7	-0.75	823	145
DEU6	GR 13034	0	9.0	22.5	-0.58	817	124
DEU7	GR 13025	41	9.6	25.6	-1.90	576	104
DEU8	GR 13023	0	9.3	23.4	-0.97	618	108
DEU9	GR 13026	30	9.7	25.5	-1.87	532	96
DEU10	GR 13027	8	9.7	25.7	-2.35	511	95
EST1	GR 13052	3.5	7.2	22.1	-4.00	565	83.7
FRA1	GR 13068	13	13.8	27.8	2.65	1,311	198
FRA2	GR 13060	525	12.2	27.7	-0.10	1,097	190
FRA3	GR 13072	1	14.8	30.2	3.05	573	51
FRA4	GR 13062	223	12.0	26.8	2.38	979	182
FRA5	GR 13067	1,200	6.6	21.1	-2.36	1,467	300
FRA6	GR 13065	911	9.5	27.2	-3.65	1,160	167
FRA7	GR 13063	800	8.9	26.1	-2.93	1,136	201
FRA8	GR 13071	50	12.0	26.3	2.20	895	128
FRA9	GR 13073	250	11.4	27.0	1.50	898	174
FRA10	GR 13061	215	11.0	27.4	-0.41	837	165
FRA11	GR 13070	297	10.0	26.3	-1.17	982	198
GBR1	GR 13078	142	9.6	22.5	0.51	663	118
GBR2	GR 13079	84	10.0	22.8	0.74	684	128
GBR3	GR 13080	134	10.7	22.4	1.38	774	148
GBR4	GR 13081	134	10.3	21.3	1.85	802	145
GBR5	GR 13082	161	10.0	19.1	2.07	968	171
GBR6	GR 13083	229	9.9	19.7	1.32	1456	229
GBR7	GR 13084	402	6.3	16.9	-1.60	1014	190
GBR8	GR 13085	402	5.3	15.4	-2.44	1047	195
LTU1	GR 13045	160	7.2	23.8	-5.59	624	105
LTU2	GR 13044	24	7.6	24.0	-5.11	637	107
NLD1	GR 13033	0	9.8	22.4	-0.10	843	136
POL1	GR 13036	2	8.9	23.4	-1.94	656	119
PRT1	GR 13075	675	11.3	28.7	-0.33	1129	92
PRT2	GR 13076	875	11.6	29.3	-0.16	1030	73
SRB1	GR 13050	1,076	6.2	22.9	-7.74	908	149
SRB2	GR 13049	1,159	6.1	22.5	-8.12	836	135
SRB3	GR 13051	149	11.7	29.8	-2.92	614	112

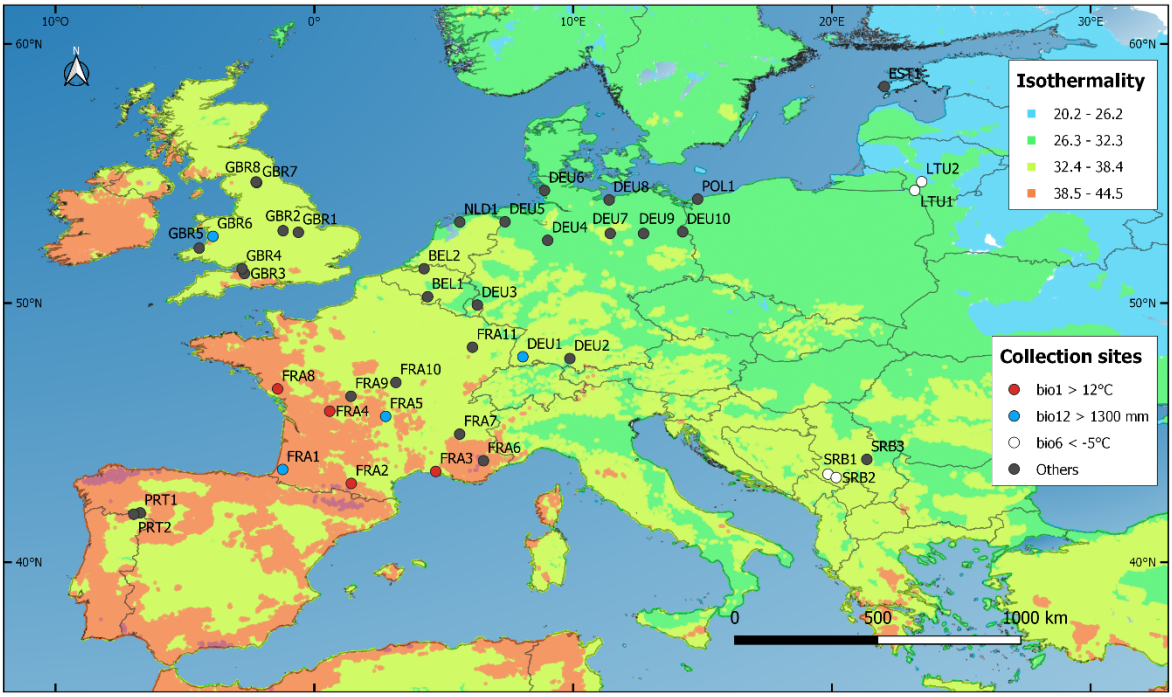

Figure 1. Collection sites of the 41 *Lolium perenne* natural populations collected across Europe in 2015. Blue sites are those with an annual cumulated precipitation norm (bio12) above 1,300mm, red sites are those with an annual mean temperature norm (bio1) above 12°C and white sites are those with average minimum mean daily temperature norm below -5°C during the coldest 14 days slice in the year (bio6). Background map colours display variation of isothermality, i.e. annual mean of temperature diurnal range (bio2)/annual range of daily mean temperature (bio7). Climate norms were computed for the period 1989–2010 (Supplemental Table 2).

Experimental design and traits evaluated

The perennial ryegrass populations, originating from various ecogeographical conditions across Europe, were cultivated in a field trial at the Satellite Collections North station in Malchow/Poel in northern Germany (Longitude 11°28'26"E, Latitude 53°59'40"N, 10masl). The site is characterized by a mean annual precipitation of 544mm and a mean annual air temperature of 10.0°C over the past 10 years. The predominant soil type is a sandy loam. The trial was set on a 38m × 18m field plot with homogeneous soil conditions. Plants were transplanted to the field and arranged in a completely randomized design on 24 September 2015 (except for plants from populations collected in Great Britain, which were transplanted on 23 October 2015 and for populations DEU8 and DEU10, which were too weakly developed and were transplanted in March 2016). One clone of each originally sampled individual plant was randomly distributed in the field trial area without replication, except three plants per population, which were set up in two replicates (clones). A number of clonal replicates were planted in April 2016, as indicated in Figure 2, because they were too weakly developed in September 2015. Spacing between the individual plants was 45 × 50cm. An equalisation cut was made at the beginning of the growing season in 2016 after recording the winter damage score. Three (2016) and four (2017) harvest cuts were completed, and fertilizers were applied after each cut, amounting to 240kg N/ha per year as calcium-ammonium-nitrate (Table 2). A weather station located at the Satellite Collections North recorded the local weather conditions at the experimental site (Supplemental Table 3). The first winter after planting was characterized by mild temperatures in November and December (7.7 and 7.1°C average daily temperatures, respectively). January was cold, with an average monthly temperature of -0.6°C (long-term average (1991–2020): 1.5°C in January in Kirchdorf/Poel (DWD, 2023a)) and 11 days with minimal temperatures below -5.0°C, followed by a very dry spring with only 9.1mm rainfall in April 2016 (long-term average (1991–2020): 32.0mm in April in Kirchdorf/Poel (DWD, 2023b)). The experimental year 2017 was characterized by average air temperatures but high rainfall in June and July (Supplemental Table 2).

The following traits were visually scored on a scale of 1 to 9 by an experienced genebank worker during the three experimental years of the trial: winter damage (WID), growing in spring (GSp), summer growth (GSu),

growing before winter (GWi) and general disease susceptibility (DIS) (Table 2). Growth parameters were assessed from a visual impression of the aboveground biomass of the plant three times in each year: in April (GSp), in August (GSu) and before winter in October (Gwi) expressed on a scale from 1 (low biomass) to 9 (strong biomass). WID describes the extent of the visually detectable damage after winter (proportion of dead tissues) on a scale from 1 (low damage) to 9 (strong damage). DIS rates the severity of the disease occurrence on the basis of visually detectable leaf damage from 1 (low susceptibility) to 9 (high susceptibility). This score gives an overall visual impression of disease infection and does not differentiate between pathogens. Heading date (HAE) of plants was recorded in 2016 and 2017 as the number of days from the first of April to the day when the tips of five ears were visible. Natural plant height was measured in spring (SPH) before the first cut using a herbometer (HerboMETRE Electronique, Framstore, France). Plant height was measured again about 10 days after the first cut and is referred to as regrowth (REG_1), i.e. the capability to recover quickly and to develop new biomass after cutting. Plant height was measured again before and after the second cut in July. The plant height 10 days after the second cut (REG_2) now gives information about the regrowth capability after the summer cut. Height increment (HgtIn) was calculated by subtracting REG1 from the height measured before the second cut (HSC) and serves as a measure of the strength of biomass growth between the cuts. Finally, tussock size was measured several times throughout 2016 and 2017 using a ruler. Tussock size was calculated as the mean of two perpendicular measurements of plant width; the highest value over recorded dates in 2016 and 2017 was used for data analysis (Table 2).

The number of living plants per population was counted 50 days after first planting date and at four later time points: (1) in spring 2016 (date of record of GSp in 2016) in order to determine survival after the first winter, (2) after heading and first cut (date of record of REG1 in 2016), (3) in spring 2017 (date of record of GSp in 2017) to determine survival after the second winter and (4) in spring 2018 (date of record of GSp in 2018) as an indicator of persistency. The number of living plants at the different record dates was expressed as a percentage of the number of plants counted 50 days after the first planting date to exclude weak plants that died right after the planting, plus later planted individuals.

Statistical analysis

The Software R (R Core Team, 2020) was used for statistical analyses. Data processing only included plants surviving the second experimental year and populations with more than 15 surviving plants. This number is based on our considerations of finding a compromise between the minimum number of plants that must have survived to calculate a reliable population mean, and ensuring that the variance model does not become too unbalanced. At the same time, we wanted to avoid excluding too many populations from further analysis, as this would result in the loss of information. Therefore, we set a threshold of at least 15 surviving plants per population, corresponding to at least 50% of the total plants/genotypes.

To test for outperforming populations, a one-way analysis of variance (ANOVA) with population as fixed effect was applied for each trait in each year. Data across all populations were visually checked for normal distribution using Q-Q-plot and the Lillie test (Gross and Ligges, 2015) and for variance homogeneity using the Levene test of the package 'car' (Fox and Weisberg, 2011). Population means were estimated with the 'lsmean' function and compared to the grand mean using the command 'eff' (Lenth, 2016). Populations were considered significantly different from the grand mean if $p \leq 0.05$. If variance homogeneity was violated, the vcovHC function (Heteroscedasticity-Consistent Covariance Matrix Estimation) of the package 'sandwich' (Zeileis, 2004) was applied for post hoc comparison.

For multivariate analysis, for each trait, population means were standardized (i.e. centred and reduced). Euclidian distances between populations and between traits were calculated using the standardized population means and the 'dist' function of base R. Clustering of the populations based on the recorded phenotypic traits was visualized in a heatmap generated with the 'heatmap.2' command of the 'gplots' package (Warnes *et al*, 2020).

Values of populations for agronomic traits were correlated to values of bioclimatic variables and geocoordinates at their sites of origin and with each other (Pearson's correlation coefficient of the R package 'Hmisc' (Harrell, 2021)). Correlations were considered significant if $p \leq 0.05$. Climate norms (period 1989–2010) of bioclimatic variables similar to the BIOCLIM variables usually derived from the WorldClim – Global Climate Database, were used. They were already presented in Blanco-Pastor *et al* (2021) and are also described in Supplemental Table 2. Fifteen BIOCLIM-like variables were included in the correlation analysis.

Results

Survival rate

In all populations, the plants survived the first winter without major losses as indicated by the high survival rates in spring 2016 (date of record of GSp_2016), which ranged from 96 to 100% (Figure 2). Of the additional, later planted individuals of DEU5, GRB1-4, GRB6 and SRB1-3, all but one each of SRB1, SRB2 and SRB3 survived until the end of the trial. No survival rate is available for the first winter for populations DEU8 and DEU10, because the respective genotypes could not be planted before March 2016. The highest losses of plants were recorded after the first cut of 2016. The survival rate at the date of record (REG1_2016) clearly declined

Table 2. Overview of main technical operations and trait records during the field experiment conducted from 2015 to 2018 to assess perennial ryegrass populations collected across Europe.

Trait[a]/Management[b]	Date of realization			Scale/Comment
	2016	2017	2018	
[a]Winter damage (WID)	02 Mar	02 Mar	19 Mar	low (1) to strong (9) damage
[b]Equalisation cut	04 Mar	09 Mar	-	
[b]Fertilizer application	04 Mar	09 Mar	-	80kg N/ha (CAN)
[a]Growth in spring (GSp)	22 Apr	20 Apr	23 Apr	low (1) to strong (9) biomass
[a]Plant height spring (SPH)	25 Apr	26 Apr	-	mm
[a]Heading date (HAE)	April–May	April–May	-	days from 1^{st} of April
[b]First cut	06 Jun	06 Jun	-	mm
[b]Fertilizer application	06 Jun	09 Jun	-	60kg N/ha
[a]Regrowth (REG1)	13 Jun	19 Jun	-	mm
[a]Height increment (HgtIn)	18 Jul	10 Jul	-	mm, HgtIn = HSC-REG1
[b]Height + Second cut (HSC)	18 Jul	17 Jul	-	mm
[b]Fertilizer application	18 Jul	17 Jul	-	60kg/ha
[a]Regrowth (REG2)	25 Jul	25 Jul	-	mm
[a]Growth in summer (GSu)	23 Aug	15 Aug	-	low (1) to strong (9) biomass
[a]Diseases (DIS)	18 Oct	04 Sept	-	low (1) to high (9) susceptibility
[b]Third cut	25 Aug	04 Sept	-	
[b]Fertilizer application	25 Aug	04 Sept	-	40kg/ha
[a]Growth before winter (GWi)	17 Oct	16 Oct	-	low (1) to strong (9) biomass
[b]Fourth cut	-	16 Oct	-	
[a]Tussock size (TUSh)	max 2016	max 2017	-	mm, mean of perpendicular width measurements

for some populations to less than 70%, but these populations then stabilized, with no further dramatic losses in the following years. The populations EST1 and LTU1 showed the highest losses at this timepoint, followed by FRA1, FRA2, PRT2, FRA3, DEU1, FRA7 and SRB1. A high survival rate at the end of the 3-year experiment was recorded for populations FRA8, DEU2, DEU5, DEU7, DEU9 and NLD1, of which $>$ 95 % of the cultivated plants survived (Figure 2).

Phenotypic characterization of the populations

The phenotypic diversity between populations was analyzed by a doubled cluster analysis on recorded traits and on populations. Eleven phenotypic traits were measured in two or three consecutive years, resulting in 24 combinations of traits and year of record (Table 2, Supplemental Tables 4 and 5). The cluster analysis on standardized population means grouped these 24 trait $\times$ year combinations into five major groups (Figure 3). The most outstanding phenotypic cluster E contained the ordinal trait winter damage recorded in all three years. Heading date and height increment between the first and second cut for both years grouped together in cluster D, while the two disease susceptibility ratings formed a separate cluster C. Plant height in spring and growth in spring in 2016 were in cluster B together with growth in summer and winter and tussock size (GSu, GWi, TUSh). The plant height in spring in 2017 and growth in spring in 2017 and 2018 formed cluster A together with the two measured regrowth heights in 2016 and 2017.

Two populations with low survival rates (EST1 and LTU1) were not included in the cluster analysis. The 39 remaining populations were assigned to five main clusters (Figure 3). Cluster 1 contained eight outstanding populations predominantly originating from Germany but also from France and Belgium. These were characterized by exceptionally low winter damage and strong and vigorous growth as indicated by high values (dark red colour) for these variables (SPH, GSp, REG, TUSh, GSu, and GWi, Figure 3). Population DEU2 was close to this cluster but had a later heading date (HAE) and a lower height increment (HgtIn). All the other populations were grouped along another branch of the clustering tree. An outstanding population was FRA3 with high winter damage and low values for the other variables. Cluster 2 (six populations from the north-eastern and central regions of the sampling area across Europe) and Cluster 3 (nine populations including five populations from Great Britain) differed for growing in spring and plant height in spring in 2016, but revealed no clear pattern for the other traits. SRB2 and SRB3 were separated from them by less vigorous growth but higher disease susceptibility and higher winter damage. Cluster 4 contained one British and six French populations and showed high winter damage (Cluster E) which was compensated for by a high spring and summer growth (GSp, GSu, Cluster A). All five populations belonging to Cluster 5 showed relatively high winter damage and low performance in all the other traits (Figure 3).

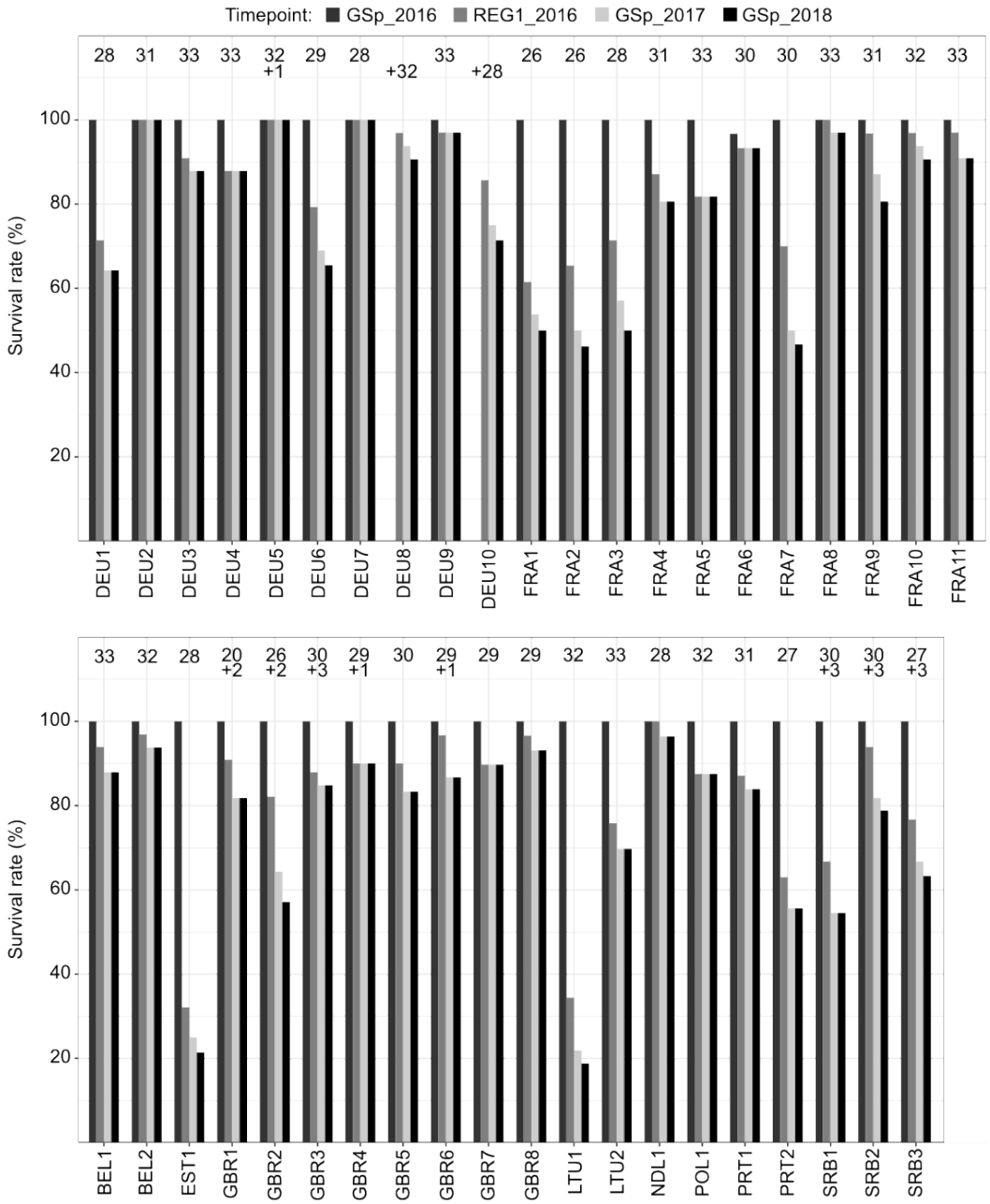

Figure 2. Survival rate (in %) of populations calculated from the number of living genotypes 50 days after planting plus later planted individuals (number of clonal replicates given at the top) and from the number of living plants at the dates when growing in spring (GSp_2016, GSp_2017, GSp_2018) and regrowth after first cut in 2016 (REG1_2016) were scored.

Winter damage, growth in spring, growth before winter and heading date presented almost similar value ranges in the three trial years, while growth in summer and disease susceptibility fairly differed between the years (Table 3). With a year-dependent average score of 5.4 to 6.1, winter damage was on an intermediate level, with the strongest damage after winter 2017. Data from the three consecutive years indicated that populations from France generally had the highest winter damage in 2016 and 2017, in particular populations originating from southern France (FRA1-FRA4) (Figure 3, Table 3, Supplemental Table 4). Populations from Great Britain (GBR3, GBR7), Serbia (SRB2, SRB3) and Portugal (PRT2) also showed high winter damage. Populations with the lowest winter damage originated from Germany, but also from Belgium (BEL2), France (FRA11) and Lithuania (LTU2). The mean value of growth in spring ranged from 4.2 to 4.8 according to years. Populations with strong growth in spring were BEL2, FRA8 and several German populations, in particular DEU9. In contrast, the material from Serbia exhibited low spring growth, particularly in 2017 and 2018. Related to their late planting in 2015, also the British populations showed low spring growth. Population FRA3 differed from all the other populations by revealing the highest winter damage and the lowest growth in spring in all three experimental years.

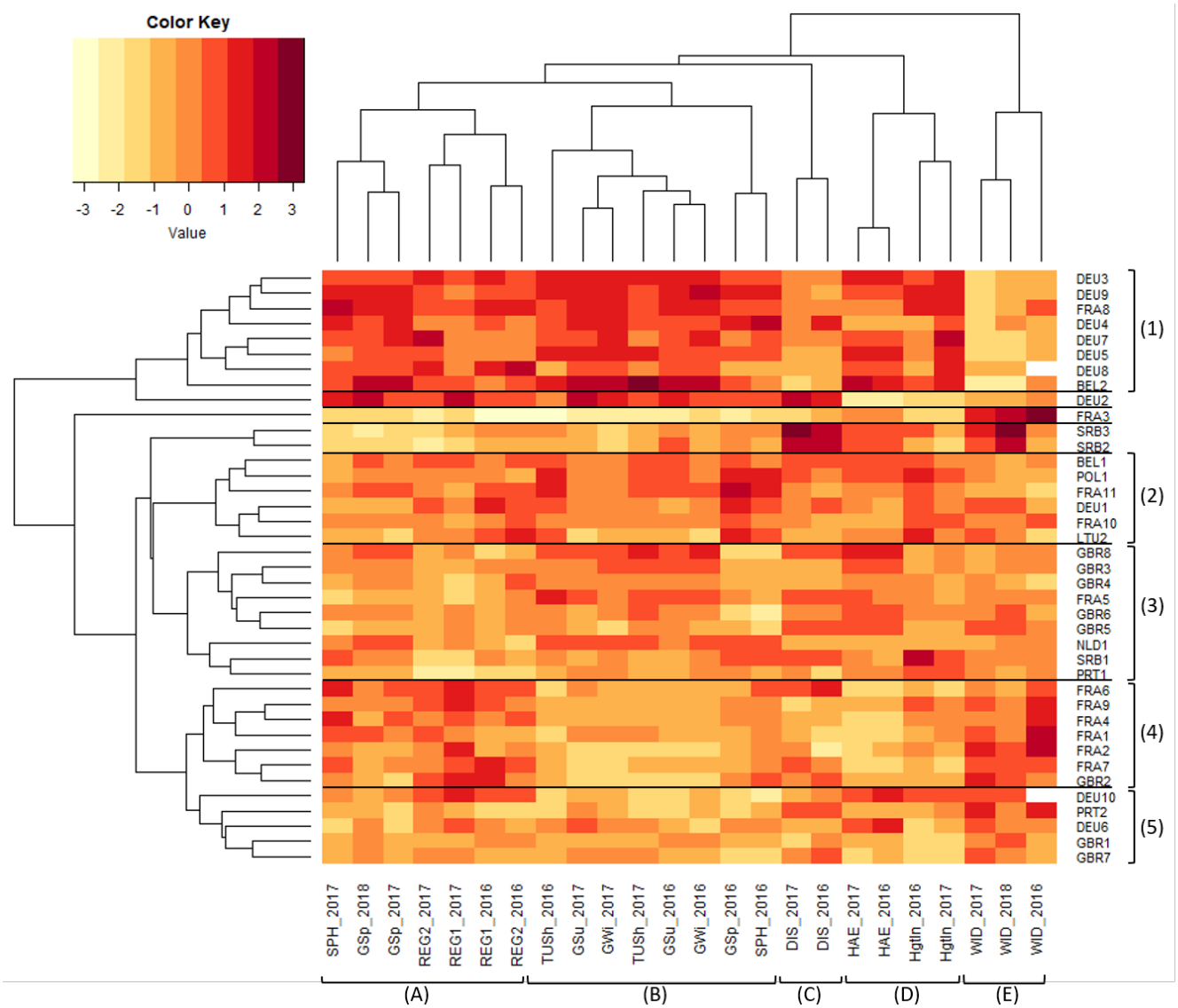

Figure 3. Clustering and heatmap based on the mean values of 11 different traits recorded in 39 natural *L. perenne* populations in two to three consecutive years under field conditions. Traits were standardized prior to multivariate analysis and are displayed for each population ranging from low (= yellow) to high (= dark red) compared with other populations. WID, Winter damage; GSp, Growth in spring; GSu, Growth in summer; GWi, Growth before winter; DIS, Disease susceptibility; HAE, Heading date; SPH, Spring height; REG, Regrowth height; HgtIn, Height increment; TUSh, Tussock size (highest). Clusters A-E grouped traits according to column means and Clusters 1-5 grouped populations according to row means, both using Euclidean distances.

Growth in summer, i.e. the biomass development of the plants in August after the two cuttings, was stronger in 2016 than in 2017. Outstanding populations with very strong growth in summer were BEL2, FRA8, DEU2, DEU3 and DEU9. Growth before winter reached rather similar values in both years. Well-developed plants with a high growth performance before winter were observed in populations BEL2, FRA8, DEU2, DEU3, DEU7 and DEU9. The average heading date was 47.0 and 48.5 days in the two years. Late types (HAE > 57 days) originated from Belgium, in particular, BEL2 with 67 days in 2017, northern Germany (DEU5-10) and northern Britain (GBR8). Although collected close to the site of GBR8, heading date of GBR7 was much earlier. Next to several French populations, DEU2 revealed the earliest heading date in the whole set with only ~25 days after 1 April in both years. General disease susceptibility, scored in September or October, reflected the general leaf health under the infection with several diseases. On average, the disease infestation was higher across populations in 2016 than in 2017. In particular FRA6, DEU2 and two out of three populations from Serbia (SRB2 and SRB3) showed a higher disease susceptibility than the other populations, even in 2017 when disease pressure was lower. Generally, based on all scored traits, BEL2, FRA8, DEU3 and DEU9 exceeded the performance of all other populations. Additionally, DEU2 revealed good biomass scorings throughout the experimental time, but tended to a higher disease susceptibility. DEU2 and DEU3 were both among the best populations for all scored traits, but strongly differentiated in heading date, with DEU2 as an early heading type and DEU3 as a late type

with an average of 60 days to heading. The weakest population was FRA3, characterized by the strongest winter damage, the lowest spring and summer growths as well as the least developed plants before winter (Supplemental Table 4).

Plant height and diameter were measured at several time points during the experiment. On average across populations, plant height in spring, regrowth height after first cut and tussock size were higher in 2017 than in 2016, while regrowth height after second cut had similar average values in the two years. In contrast, height increment, as a measure of plant height development between first and second cuts, was only about half as high in 2017 as in 2016 (Table 3). The spring height measurements confirmed the findings from growth in spring scorings. The average plant height in spring was 125mm in 2016 and increased to 205mm in 2017. Populations with large spring heights were mostly from France (FRA4, FRA6, FRA8, FRA11) and Germany (DEU2, DEU4, DEU8, DEU9). Similar to growth in spring scorings, spring height was exceptionally low for all the material from Great Britain, particularly in 2016, and for material from Serbia (Figure 3, Supplemental Table 5). Regrowth height 10 days after the first cut reached 137mm on average across populations in 2016 and 196mm in 2017. Populations with strong regrowth after the first cut were FRA7, FRA8, FRA9 and DEU1, DEU2, DEU3, DEU8 and LTU2. Populations from PRT and SRB generally revealed a slow regrowth after the first cut. The regrowth height 10 days after the second cut was as on average 183mm and rather stable between the two years. The population with regrowth height after second cut stronger than most of the other populations, considering both years, originated from Germany (DEU3, DEU5, DEU7, DEU8, DEU9) and France (FRA8). In contrast, regrowth capability below that of most of the other populations was measured for populations from Portugal (PRT1), Serbia (SRB2) and France (FRA3). The increment of plant height, expressed as the height difference from regrowth height after first cut to the height at the second cut, declined on average across populations from 147mm in 2016 to 92mm in 2017. Outstanding populations with a high height increment in both years and a late spring growth were DEU9 and FRA8. Tussock size of the individual plants averaged 206mm at the end of 2016 and increased on average by 50mm by the end of 2017. Highest tussock sizes ($>$ 290mm) were generally recorded for the strong growing populations such as BEL2, FRA8, DEU3, DEU4, DEU5 and DEU8, while most populations from France, Portugal and Serbia had tussock sizes below 230mm.

Relation between performances of populations and bioclimatic variables at their sites of origin

Correlations between the phenotypic traits of the populations and the climatic variables at their sites of origin are displayed in Figure 4. Among all phenotypic traits, winter damage in 2016 highly correlated with almost all climatic variables. Winter damage in 2016 showed the highest correlations with the mean annual temperature (bio1, $r = 0.59$, $p < 0.001$) and mean temperature in the driest quarter (bio9, $r = 0.67$, $p < 0.001$). The temperature daily range correlated with the susceptibility of the populations to cold winters as indicated by the correlation between winter damage in 2016 and temperature diurnal range (bio2, $r = 0.45$, $p < 0.001$). However, correlations were weaker and not significant in the following years. Growth in spring correlated negatively with minimum temperatures during cold seasons (bio11, $r = -0.40$, $p < 0.05$), but only for 2016. Hence, the lower the temperature during the winter period at the site of origin, the stronger was the plant growth in spring after transplanting. Furthermore, temperature seasonality (bio4) was positively correlated with growth in spring 2016 with $r = 0.42$ ($p < 0.01$). Days to heading correlated little with bioclimatic variables, except for mean diurnal range in both experimental years (bio2, $r = -0.46$, $p < 0.01$/-0.43, $p < 0.01$, Figure 4).

Tussock size in both years correlated negatively with bio2 ($r = -0.40$, $p < 0.05$/-0.55, $p < 0.001$). Disease susceptibility in both years correlated negatively with several temperature variables but not with precipitation indices. The lower the average daily minimum temperature (bio6, $r = -0.53$, $p < 0.001$/-0.57, $p < 0.001$) and the lower the mean temperature of the coldest quarter (bio11, $r=-0.51$, $p<0.001$/-0.48, $p < 0.01$), the higher was the damage caused by diseases in the field experiment. Accordingly, the higher the altitude of the site of origin, the higher the disease susceptibility in both years ($r = 0.48$, $p < 0.01$/0.53, $p < 0.001$). The latitude of the site of origin correlated positively with heading date, growth before winter and tussock size and negatively with winter damage in 2016 and 2017. Other phenotypic traits showed only low correlations with the basic climate norms (Figure 4).

Discussion

We studied the agronomic performances of perennial ryegrass populations collected across Europe and found large differences between the populations at the study site in northern Germany. Winter damage was high for accessions from warm and sunny origins with dry summers, as seen by positive correlations between winter damage and temperature (bio1, bio9, bio10) and negative correlations between winter damage and precipitation in the warmest quarter (bio18). Especially populations from southern European regions with a warm climate at the site of origin, such as FRA1-FRA4 and PRT2, showed high winter damage, followed by low spring growth and, finally, a high loss of plants after the first cut in 2016. This is consistent with previous results. Keep *et al* (2021) and Blanco-Pastor *et al* (2021) reported that perennial ryegrass populations collected from areas with low winter temperatures were more resistant to winter damage in cold winters, while populations adapted to long and dry summers

Table 3. Phenotypic traits measured in 39 natural *L. perenne* populations in two or three consecutive years under field conditions. Given are average, minimum and maximum values of populations and populations significantly different from the grand mean. *, Populations significantly higher than the grand mean for the traits GSp, GSu, GWi, SPH, REG1, REG2, HgtIn, TUSh; populations significantly lower than the grand mean for WID and DIS; HAE: the five significantly earliest populations and the five significantly latest populations; significance level $p \leq 0.05$; see more detailed results in Supplemental Tables 4 and 5.

Trait	Year	Mean	Min	Max	Important populations*
Winter damage (WID) [Score 1-9]	2016	5.8	4.8	8.3	Low: DEU9, FRA11, GBR4, LTU2
	2017	6.1	4.7	7.4	Low: BEL2, DEU3, DEU4, DEU5, DEU7, DEU8, DEU9, FRA8
	2018	5.4	4.2	6.9	Low: BEL2, DEU5, DEU7
Growth in spring (GSp) [Score 1-9]	2016	4.5	2.6	6.2	High: BEL2, DEU1, DEU3, DEU4, DEU5, DEU9, FRA8, FRA11
	2017	4.8	2.9	6.6	High: BEL2, DEU4, DEU7, DEU9, FRA8
	2018	4.2	2.7	5.5	High: BEL2, DEU2, DEU9, FRA8
Growth in summer (GSu) [Score 1-9]	2016	5.9	3.2	8.2	High: BEL2, DEU2, DEU3, DEU9, FRA8
	2017	4.3	2.9	5.8	High: BEL2, DEU2, DEU3, DEU5, FRA8
Growth before winter (GWi) [Score 1-9]	2016	4.7	2.9	6.4	High: BEL2, DEU3, DEU7, DEU9, FRA8
	2017	5.0	2.3	6.9	High: BEL2, DEU2, DEU3, DEU4, DEU5, DEU7, DEU9, FRA8
Disease susceptibility (DIS) [Score 1-9]	2016	5.7	4.7	7.0	Low: -
	2017	3.9	2.9	5.6	Low: BEL2
Heading time (HAE) [Days from 1April]	2016	47.0	25.8	63.3	Early: DEU2, FRA6, FRA7, FRA1, FRA4, late: BEL2, GBR8, DEU10, DEU5, DEU6
	2017	48.5	25.5	61.1	Early: DEU2, FRA6, FRA7, FRA1, FRA4, late: BEL2, GBR8, DEU10, DEU5, DEU6
Spring height (SPH) [mm]	2016	125	82.5	164	High: DEU4, DEU9, FRA8, FRA11, NLD1, POL1, SRB1
	2017	207	159	264	High: DEU2, DEU4, DEU8, DEU9, FRA4, FRA6, FRA8
Regrowth height 1 (REG1) [mm]	2016	137	105	157	High: DEU1, DEU2, DEU3, DEU8, FRA7, FRA8, FRA9, GBR2, LTU2
	2017	196	165	222	High: DEU2, FRA6
Regrowth height 2 (REG2) [mm]	2016	183	133	112	High: DEU8, FRA8
	2017	182	156	205	High: DEU3, DEU5, DEU7, DEU8, DEU9
Height increment (HgtIn) [mm]	2016	147	103	204	High: DEU3, DEU9, FRA8, FRA10, LTU2, POL1, SRB1
	2017	92.0	57.3	129	High: DEU7, DEU8, DEU9, FRA8
Tussock size (highest, TUSh) [mm]	2016	206	122	253	High: BEL2, DEU3, DEU4, DEU5, DEU9, FRA5, FRA8, FRA11, GBR8, LTU2, NLD1, POL1
	2017	258	204	363	High: BEL2, DEU3, DEU4, DEU5, DEU9, FRA5, FRA8, FRA11, GBR3, GBR8, POL1

(and probably heat stress) revealed high aftermath heading and spike density but low persistency after cold winters. Similarly, Hulke *et al* (2007) found a higher tiller survival after cold winters in perennial ryegrass populations originating from northern, continental or alpine areas of Europe than in populations from Mediterranean areas with warmer climates. Bachmann-Pfabe *et al* (2018) also found that perennial ryegrass populations from north-western Spain were sensitive to low temperatures in winter. Reports from Bachmann-Pfabe *et al* (2018), Blanco-Pastor *et al* (2021) and Keep *et al* (2021) confirmed that populations from southern Europe with mild winter temperatures can grow in early spring and late autumn and even to some extent in winter. However, this ability makes them maladapted to the winter conditions of our northern Germany experimental field, during which their living tissues were exposed to cold and frost. An adaptation to low winter temperatures is to stop growth during winter and start growing late in spring in order to escape cold stress (Keep *et al*, 2021), which is not present in these southern European populations. In accordance with Blanco-Pastor *et al* (2021), who found late spike emergence in cold-adapted populations, winter damage correlated negatively with heading date in our study (Supplemental Figure 1).

The correlation between winter damage and climate at sites of origin was only prominent in the first winter 2015–2016, but was negligible for the other years. That might be attributed to remarkably low temperatures in January 2016 (average -0.6°C, minimal daily average temperature of -9.2°C), which differentiated the populations originating from cold regions and the cold-sensitive populations from warmer regions. In the following years, acclimatization to the environmental conditions at the study site, i.e. by sufficient hardening,

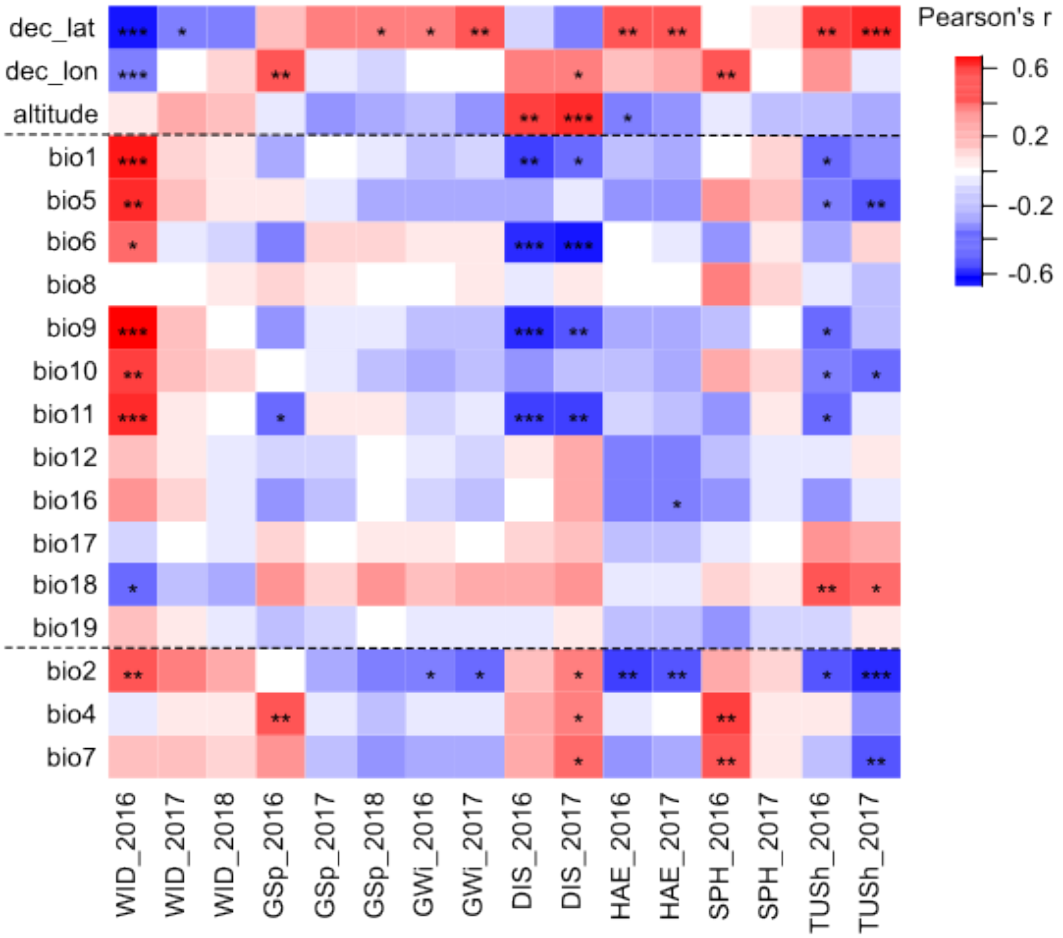

Figure 4. Correlations between origin-specific variables (latitude, longitude, altitude and bioclimatic variables) and mean values of phenotypic traits of the 39 *L. perenne* populations. Colour codes are Pearson's correlation coefficients (see sidebar on the top right). Significant correlations are labelled with asterisks (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). dec_lat, latitude; dec_lon, longitude; bio1, annual mean temperature; bio5, average maximum temperature of the warmest 14/15-day period; bio6, average minimum temperature of the coldest 14/15-day period; bio8, bio9, bio10, bio11, mean temperature of the wettest, driest, warmest and coldest quarter, respectively; bio12, annual cumulated precipitations; bio16, bio17, bio18, bio19, cumulated precipitations in the wettest, driest, warmest and coldest quarter, respectively; bio2, mean diurnal range; bio4, temperature seasonality (standard deviation of average daily mean temperature per year); bio7, temperature annual range. WID, Winter damage, GSp, Growth in spring, GWi, Growth before winter, DIS, Disease susceptibility, HAE, Heading date, SPH, Spring height, TUSh, Tussock size (highest). Only phenotypic traits with at least one correlation with $r > 0.4$ or $r < -0.4$ in at least one year are shown.

especially since only plants that survived two years were considered in the analysis, could have contributed to the lack of climate-winter damage correlations. Past climatic experience affects plant performance, as indicated by a study with 18 Arrhenatherum elatius ecotypes, where tolerance to (late spring) frosts increased when plants were exposed to late frost in the preceding growing season (Kreyling *et al*, 2012).

For European perennial ryegrass accessions, the minimum lethal temperature, i.e. the temperature at which 50% of the plants would die, was determined to be between -3.0 and -13.9°C (Humphreys and Eagles, 1988; Hulke *et al*, 2008). Hence, cold-sensitive genotypes in our experiment were not killed but probably damaged by the low temperatures in the first winter. These plants might survive freezing, but their growth is limited in subsequent spring (Humphreys and Eagles, 1988). Accordingly, populations with strong winter damage, such as FRA1-4, FRA9 and PRT2, revealed low growth in spring in our experiment. The low precipitation in March and April 2016 at the experimental site might have further contributed to a low spring growth of already winter-damaged plants and the subsequent loss of many genotypes after the first cut.

Unexpectedly, also populations SRB1, SRB3, LTU1 and EST1 showed high losses (low survival rate) after the first cut in 2016, although they originated from regions with average temperatures in the coldest month reaching -7°C. Freezing tolerance is influenced by additional factors such as hardening, wind desiccation, snow cover or disease infestation (Hulke *et al*, 2007). Hardening prior to freezing is also critical to reduce cell damage caused by frost (Humphreys and Eagles, 1988). According to Fuller and Eagles (1980), the threshold for hardening in perennial ryegrass is between 5 and 7°C for European germplasm, and between 2

and 5°C for less tolerant New Zealand cultivars, while higher temperatures may even cause de-hardening. Hence, the average temperatures of 7.7 and 7.1°C in November and December 2015 might have hampered cold acclimatization and made the plants prone to other stresses. Nevertheless, population LTU2 from Lithuania is well suited to breed for winter hardiness, showing high survival rates and persistency as well as fast growth in spring and after cut (high SPH, REG1 and REG2) including large tussock size. Also, disease infestation might reduce frost tolerance and could explain the losses in populations from Serbia. Although disease infestation was not recorded prior to the first winter (in 2015), ratings from autumn 2016 and 2017 indicate a higher disease susceptibility in Serbian populations, in particular in SRB3. Fungal diseases such as crown rust reduce the overall vigour and competitiveness of ryegrass (Kimbeng, 1999) and thus may also negatively affect winter hardiness or the tolerance to drought in early spring.

Disease susceptibility in 2016 and 2017 was negatively correlated to temperature variables (bio1, bio6, bio9, bio11), indicating that populations from cold regions were more susceptible to disease infestation. Geographical variation in the disease susceptibility of natural populations of grass species was already reported as associated with climatic variation. Germplasm with higher tolerance to rust infection was, for example, identified in perennial ryegrass material collected in Romania, Crisana region (Willner *et al*, 2010), and Bulgaria (Bachmann-Pfabe *et al*, 2018). In a study with fine-leaved fescues across France, Sampoux and Badeau (2010) found that populations from warm and wet sites were the most resistant to diseases. Climate conditions likely determine the presence/absence of pathogens, and the co-evolution of plant species with the pathogens to which they are exposed may lead to the development of resistance in plant genotypes.

Scores of biomass growth in spring, summer and before winter, are indicators of the potential fodder production of the studied populations in the different seasons. The cluster and heatmap analysis identified populations of cluster 1 (Figure 3), namely BEL2, FRA8, DEU2, DEU3, DEU4, DEU5, DEU7 and DEU9, as best combining strong growth in spring, summer and autumn, large tussock size, late heading (except DEU2) and low winter damage. These populations also showed a high survival rate throughout the experiment. These results might not be very surprising, since these populations originate from habitats with climate conditions very similar to the experimental site. They are more unexpected for population FRA8, which comes from a site with milder winter temperatures and possible occurrence of drought events in summer. Population FRA3 clearly contrasted with populations of Cluster 1 with the weakest vegetative growth, the smallest plant size and highest winter damage observed in the whole set of populations. The environment of this population is characterized by the highest winter temperature (bio6, 3.5°C), the warmest summer temperature (bio5, 30.2°C) and the lowest precipitation in the driest quarter (bio17, 50mm). Reduced growth potential (especially in summer) and plant size (small tussock size) in this population are consistent with the growth-survival trade-off under the risk of summer heat and drought, a concept published by Blanco-Pastor *et al* (2021) and Keep *et al* (2021). We also found that tussock size was positively correlated to precipitation in the wettest quarter (bio18) and negatively to maximum temperatures (bio5), mean diurnal temperature range (bio2) and annual temperature range (bio7). Thus, we observed large tussocks (and high vegetative growth) in populations from the sites where the summer drought stress is the smallest (or without summer drought stress). Wet climates with high rainfall, low summer drought stress and small diurnal and annual temperature ranges are found in oceanic regions.

Heading date is an important feature for perennial ryegrass as a forage crop. After heading, the proportion of reproductive tillers increases rapidly in the sward biomass, while the subsequent biomass growth slows down and the feeding quality decreases (Hurley *et al*, 2007). Plant breeders developed forage-type perennial ryegrass cultivars with different maturity groups, from early-heading types convenient for early-season cut to late-heading types best adapted to a long grazing season and used for permanent pastures (Laidlaw, 2005; Humphreys *et al*, 2010). Heading date is a highly heritable trait with a broad sense heritability usually larger than 80% (Keep *et al*, 2020); it was found to strongly correlate between two record years with Pearson's product-moment correlation of 0.82 in perennial ryegrass full-sib families by Arojju *et al* (2016). Our study is consistent with this well-known trend, as indicated by a correlation of 0.96 ($p < 0.001$) between heading date in 2016 and in 2017 (Supplemental Figure 1). Populations BEL2, DEU5 and GBR8 were identified as the latest heading ones, while populations DEU2, FRA6 and FRA7 were scored as the earliest heading ones. Heading date of perennial ryegrass populations was previously reported as related to the climate and the solar radiation at sites of origin. Barre *et al* (2018), Blanco-Pastor *et al* (2021) and Keep *et al* (2021) typified perennial ryegrass populations from sites with cold winter and spring by slow spring growth and late heading. That corresponds with an escape strategy in which the peak of vegetative spring growth is scheduled to escape the risk of late cold stress (Blanco-Pastor *et al*, 2021). On the other hand, early heading and fast spring growth were reported in perennial ryegrass populations from sites with favourable conditions for growth, i.e. mild winters, cool summers and abundant rainfall (Keep *et al*, 2021). Accordingly, populations such as FRA1-4, FRA9 and PRT2 with early heading date and thus early start of spring growth, were exposed to cold injuries and had high winter damage. LTU2 had few winter damages, although it is quite early heading, like FRA11 and DEU4. These populations could have acquired good winter

hardiness or may have other physiological patterns that enable them to cope with cold in winter and spring. Heading date in our experiment showed no direct relationship to temperature norms at the sites of origin. But heading date was notably negatively correlated with mean diurnal temperature range across the year and positively with latitude. Regions with large diurnal temperature ranges are usually continental regions with dry and cold climates with unfavourable conditions for growth and therefore later heading. Similarly, higher northern latitudes have lower solar radiation and are often colder.

Since the germplasm used for this study was not compared to cultivars, we cannot assess the performance level, neither can we assess the performance in swards or mixtures. However, our study confirms the importance of breeding efforts for regionally adapted plant material. In general, the adaptation to the climate conditions at the site of origin in our newly collected *L. perenne* panel followed the same trends as observed by studies evaluating *L. perenne* genebank accessions collected from 1980 to 2000 (Blanco-Pastor *et al*, 2021; Keep *et al*, 2021). Therefore, we can assume that the natural distribution of perennial ryegrass phenotypic diversity across Europe is still related to the average climate conditions that prevailed during the past decades, even if climate change has likely accelerated from the time of collection of genebank accessions to that of the newly collected ones in this study.

Conclusions

The phenotypic characterization of *L. perenne* populations originating from habitats across Europe in a common garden experiment showed that climate may be a major driver for phenotypic variation between populations. Our results report on climate adaptation of a newly collected set of ecotypic populations of perennial ryegrass. This set of natural populations collected in contrasting environments is available as genetic resources for breeding and research at the Collections for Oil and Fodder Crops of the IPK Genebank, and offers opportunities to widen the genetic base for selection. Populations from environments similar to the experimental site had a better growth performance and may be integrated directly into breeding programmes for the North of Germany, whereas others might be considered to improve specific traits.

Supplemental data

Supplemental Table 1: Collection Material. Passport data of the collected perennial ryegrass (*Lolium perenne* L.) populations including information about the sample area.
Supplemental Table 2: Bioclimatic variables used to describe the climatic conditions at the sites of origin of the perennial ryegrass populations (BIOCLIM like variables).
Supplemental Table 3: Weather conditions over the experiment duration at the field trial site.
Supplemental Table 4: Population means for the traits evaluated.
Supplemental Table 5: Adjusted means of the different populations in the field experiment from March 2016 to 2018 for the measured traits including statistics from a one-way ANOVA (F- and p-values).
Supplemental Table 6: Pearson correlation coefficients between the phenotypic traits (population means) and the climate norms at the site of origin of populations.
Supplemental Figure 1: Pearson correlation coefficients r between the phenotypic traits (population means).

Data availability

The phenotypic data recorded in this experiment and presented in this manuscript are available via the Genebank Information Systems of IPK https://gbis.ipk-gatersleben.de/gbis2i/ by typing the accession number given in Table 1 or the accession DOI given in the Supplemental Table 1. Furthermore, the data can be accessed via the EURISCO search catalogue http://eurisco.ecpgr.org. The description of the BIOCLIM-like climate variables and of the seasonal climate variables at sites of origin of populations was published by Blanco-Pastor *et al* (2021).

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Conflict of interest

The authors declare no financial or personal conflict of interest. The funders had no role in the design of the study, in the collection, analyses, or interpretation of the data, in the writing of the manuscript or in the decision to publish the results.

Author contributions

SBP analyzed the data, SBP and MK wrote the manuscript, AR and EW were responsible for the experimental design, trial management and collected the data, EW and KJD reviewed the manuscript, JPS provided data on climate norms and climate variability and reviewed the manuscript. All authors read and approved the manuscript.

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Genebank Peer Reviews: A powerful tool to improve genebank quality and promote collaboration

Theo van Hintum[a], Sharon Balding[b], Gergana Desheva[c], John Dickie[b], María José Díez[d], Luis Guasch[e], Pavol Hauptvogel[f], Vojtěch Holubec[g], Dagmar Janovská[g], Ulrike Lohwasser[h], Isaura Martín[e], Ludmila Papoušková[g], Beate Schierscher-Viret[i], Lise Lykke Steffensen[j], Katya Uzundzhalieva[c], Patrizia Vaccino[k] and José Vicente Valcárcel[d]

[a] *Centre for Genetic Resources, The Netherlands (CGN), Wageningen University and Research (WUR), Wageningen, The Netherlands*
[b] *Millennium Seed Bank (MSB), Royal Botanic Gardens Kew, Wakehurst Place, Ardingly, UK*
[c] *Agricultural Academy, Institute of Plant Genetic Resources "Konstantin Malkov" (IPGR), Sadovo, Bulgaria*
[d] *Institute for the Conservation and Improvement of the Valentian Agrodiversity (COMAV), Polytechnic University of Valencia, Spain*
[e] *National Institute for Agricultural and Food Research and Technology (INIA-CSIC), Spanish Plant Genetic Resources Centre (CRF), Alcalá de Henares, Madrid, Spain*
[f] *Research Institute of Plant Production (RIPP), National Agricultural and Food Centre (NPPC), Piešťany, Slovakia*
[g] *Czech AgriFood Research Center (CARC), Ruzyně, Prague, Czech Republic*
[h] *Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany*
[i] *AGROSCOPE, Changins, Nyon, Switzerland*
[j] *Nordic Genetic Resource Center (NordGen), Alnarp, Sweden*
[k] *Research Center for Cereal and Industrial Crops (CREA-CI), Council for Agricultural Research and Economics (CREA), Vercelli, Italy*

Abstract: The conservation of plant genetic resources (PGR) is critical to ensuring global food security and agricultural sustainability. Genebanks play a vital role in *ex situ* conservation, complementing *in situ* strategies by preserving crop diversity (incl. their wild relatives) and providing access to biological materials for research, breeding and farming. However, maintaining high conservation standards and ensuring accessibility remains a global challenge. To address this, the 'Genebank Peer Review' system was developed as a collaborative quality assessment and improvement mechanism. This system facilitates reciprocal evaluations among genebanks, promoting transparency, capacity building and continuous improvement in conservation practices. Implemented in Europe since 2019, the peer review process involves structured self-assessments, site visits and expert evaluations, culminating in publicly available reports that guide genebanks in enhancing their operations. Feedback from participating institutions highlights the system's effectiveness in fostering knowledge exchange, strengthening professional networks and improving genebank management practices. Despite its success, challenges remain, particularly regarding expert availability and resource constraints. Future efforts should focus on institutionalizing mentorship programmes to sustain and expand the impact of Genebank Peer Reviews and monitor improvements.

Keywords: crop diversity, *ex situ* conservation, plant genetic resources, peer review, quality improvement

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Introduction

To ensure a sufficient food supply for the growing global population, it is crucial to conserve plant genetic resources (PGR) and make them accessible for crop research, plant breeding and cultivation, both now and in the future. Since the 1960s, genebanks have been established to complement traditional *in situ* conservation methods (Engels and Ebert, 2021). This shift from *in situ* to *ex situ* was necessary due to changes in agriculture, land use, and environmental conditions that threatened to erode the *in situ* biodiversity.

Genebanks offer several advantages over *in situ* conservation, including improved access to both information and biological material. However, the task of conserving PGR is huge, and no single genebank or country can manage it alone. It requires a global effort with contributions from various actors. Many research institutions involved in plant breeding have established genebanks, and most countries maintain a national genebank or a network of genebanks, often linked to national breeding programmes. According to the Food and Agriculture Organization (FAO, 2025), in 2022 the global genebank network conserved approximately 5.9 million accessions across 871 genebanks composed of 13 international genebanks (such as those managed by CGIAR), 6 regional and the remaining 852 national genebanks – more than half of them in Europe.

The global effort to conserve PGR is supported by international treaties and collaborations, such as the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA, (FAO, 2009)), which aims to ensure the conservation and sustainable use of PGR and the fair sharing of benefits arising from their use. This collaborative approach is essential for maintaining the genetic diversity needed to adapt to future agricultural challenges and ensure food security.

Obviously, not every genebank intends to contribute to the 'global effort'. Included in the 871 genebanks are many local – what could be called 'working collections', not meeting any standards (FAO, 2014) in terms of conservation practice or access. For example, only 123 out of the 852 genebank deposited seeds at the Svalbard Global Seed Vault (https://www.seedvault.no/). This could be due to well-considered decisions, but in most cases, it may be a lack of awareness, with resulting shortcomings in procedures.

To be considered part of the 'global effort' a genebank should have adopted two principles: (1) the material should be properly conserved and (2) the material should be accessible for use.

The first principle, proper conservation, is a technical issue. Obviously one can disagree about the technical details, such as the frequency of germination tests, the population size of regenerations, and the need to triplicate at the Svalbard Global Seed Vault, but the objective is clear. Technical disputes are about the balance between security (genetic integrity and seed quality) and costs: the higher the security, the higher the costs per unit and as a result the lower the number of units that can be conserved.

The second principle, access, is not only technical, but also has a political dimension. On the technical side, one could ask what information should be provided about the material, what percentage of the material conserved should be readily available for distribution, or what a genebank should be willing to do, to get the material to the requestor (in terms of plant passports, import permits, non-GMO statements, etc.). On the political side, it can be more complex. Considerations will have to take account of access and benefit-sharing policies: who is allowed to receive material and under what conditions?

Fortunately, there is a broad base of experience to draw on. Regarding the conservation issues, there are the FAO Genebank Standards (FAO, 2014) and extensive experience from genebanks. Regarding the access issues, there are the ITPGRFA, the EU regulation on the Nagoya Protocol (EU, 2014) and organizations such as the European Cooperative Programme for Plant Genetic Resources (ECPGR) setting standards or proposing best practices (https://www.ecpgr.org/).

In the domain of genebank performance monitoring, a certain level of experience has been accumulated within the community. A number of genebanks have adopted the ISO 9001 standard for quality management, thereby ensuring adherence to established protocols, effective risk management, and the implementation of continuous quality improvement mechanisms, including procedures for addressing user feedback and complaints. Additionally, the Global Crop Diversity Trust has developed the Genebank Quality Management System (GQMS) and has provided support for its implementation across CGIAR genebanks (Lusty *et al*, 2021). Nevertheless, the establishment and maintenance of such systems entail substantial investments, both from the genebanks themselves and from the entities responsible for review and auditing (see also van Hintum and Wijnker (2024)). In a situation where the genebank community has community standards, the challenge is to ensure that the members of this community meet those standards, especially where funds are scarce. Some genebanks will already meet the standards to a certain extent, while others will need changes in protocols or even additional equipment or staff. In the attempt to improve the quality of operations in the community, the obvious first step is to determine the status quo. The members of the community, the genebanks, need to determine how they are doing, and identify where improvements can or should be made. For this reason, the 'Genebank Peer Review' system was designed to create clarity and transparency about the status of genebanks and to build capacity to improve collections' status where needed and possible. Initially, this peer-review system is aimed at the European national genebanks wanting to be part of the global effort to conserve PGR for future generations and make it available to the current.

History of the Genebank Peer Review system

The PGR community in Europe is very heterogeneous, consisting of hundreds of genebanks (FAO, 2025), with a huge range of objectives, sizes and methodologies. Ideally, Europe could set up a system similar to the USDA National Plant Germplasm System (https://www.ars-grin.gov/npgs/), i.e. including some central and some sub-regionally specialized facilities, with proper coordination, quality management and a clear policy regarding access. ECPGR, as the collaborative umbrella organization for European countries, has made efforts to address the heterogeneous and fragmented landscape but has yet to achieve significant improvements in coordination. Initiatives such as AEGIS (www.ecpgr.org/aegis), a catalogue of PGR managed according to standards in various participating European genebanks, were promising but still need major steps forward to become effective (van Hintum *et al*, 2021).

During an ECPGR meeting about 'Assessing current practices and procedures to strengthen AEGIS' held in Madrid in December 2018, a plan was presented to improve transparency and build capacity in the European genebank community based on mutual visits of genebank staff members (Engels *et al*, 2019). This plan – called 'Improving PGR Conservation and Access in Europe; a plan to create a voluntary genebank's peer review system' – was prepared and presented by the Centre for Genetic Resources, The Netherlands (CGN), based on its involvement in the reviewing process organized by the Global Crop Diversity Trust coordinating and supporting the operations of the CGIAR genebanks (Lusty *et al*, 2021). The plan was received positively and it was decided that: "Peer review proof of concept will be tested (colleague experts visiting, discussing and learning from each other – not an auditing or policing exercise)" and that "Under the coordination of CGN, first peer-review cycle will be completed in early 2019".

In the first months of 2019, CGN formulated protocols for peer reviews, including elements of AQUAS, the quality management system of AEGIS (ECPGR, 2025a). In the period from February to April 2019, a pilot cycle of peer reviews was organized, partly in the framework of the EU project GenRes Bridge (https://www.genresbridge.eu/). This project aimed "to strengthen conservation and sustainable use of genetic resources by accelerating collaborative efforts and widening capacities in plant, forest and animal domains". The pilot cycle involved the IHAR-BIP genebank in Radzików, Poland, the COMAV genebank in Valencia, Spain and CGN in Wageningen, The Netherlands. After each visit a short report was written with observations and recommendations, and after the complete cycle, a summary of experiences and conclusions was written and made public on the ECPGR website (van Hintum *et al*, 2019).

Based on this pilot cycle of reviews, the protocols for the peer reviews were further improved and the concept was used in another EU-funded project, AGENT (Activated Genebank Network, https://agent-project.eu/). This project aimed "to unlock the full potential of biological material stored in gene banks worldwide by utilizing FAIR international data standards and an open digital infrastructure for managing plant genetic resources". In this context another four cycles of reviews were organized in the period 2022–2024, with 12 reviews involving eleven genebanks; CGN was involved in two cycles.

The concept of Genebank Peer Reviews

The fundamental principle of Genebank Peer Reviews is straightforward: within a cycle of three reciprocal visits, experienced staff members from two genebanks evaluate the facilities and operations of a third institution. These visits are preceded by a self-assessment conducted by the host genebank, the results of which are made available to the reviewers. During the review process, the visiting experts are granted full access to relevant information. Following the evaluation, the reviewers compile a report, which is subsequently published online, contingent upon the consent of the reviewed genebank. Further details, primarily based on the GenRes Bridge report on this activity (GenRes Bridge, 2021), are provided below.

Objectives

The primary objective of the peer review process is to provide a comprehensive description of the management, facilities and procedures of a genebank and evaluate them. This serves two key purposes: (1) enabling expert colleagues to offer technical feedback on these aspects, and, when necessary, (2) facilitating the development of an expert-driven improvement plan. Such an improvement plan, informed by the review report, may be utilized in fundraising efforts. The transparency fostered by these peer reviews is expected to benefit the PGR community by identifying both strengths and weaknesses within the PGR management infrastructure. This, in turn, will allow for the optimization of strong points and the implementation of targeted measures to address areas requiring improvement.

The peer-review process aims to assess all pertinent aspects of the genebank under evaluation, including management, facilities and procedures, within a 2-to-3-day visit.

Preparation

The initial phase of the process entails identifying appropriate partners and systematically planning the reviews, as detailed below:

- Three participating genebanks are selected. This selection is based on the context of the reviews, i.e. the genebanks participating in the project that organizes the peer reviews, and obviously the enthusiasm of the genebanks to be included.
- Each genebank designates a qualified representative who will actively participate in all three

reviews. This individual should possess extensive knowledge of their respective genebank and expertise in PGR management. The representative will serve as the primary contact throughout the review process. In exceptional cases, a genebank may appoint two representatives if warranted.
- Tentative dates for the three reviews are determined, with each review expected to last between two and three days, depending on the complexity of the operations.

For each review:

- The reviewed genebank provides, when available, technical information relevant to its operations, including management structures, facilities and procedures. This is achieved through the completion of the Operational Genebank Manual, which should be compiled using the template provided by ECPGR (2010), or a version slightly expanded version for the peer reviews (see Supplemental Material 1).
- Reviewers may request specific data to facilitate their preparatory work.
- The reviewed genebank prepares a draft agenda, allowing reviewers to provide feedback and propose amendments beforehand.
- The reviewed genebank arranges accommodation for the reviewers and covers the associated costs.
- Reviewers organize and finance their own travel to the genebank's country.

Review process

- The reviewed genebank assumes responsibility for organizing local transportation during the review and covering related expenses.
- The reviewers are granted full access to the genebanks records, staff and facilities upon request. Should any restrictions apply, these must be formally documented along with a rationale.
- Throughout the visit, the reviewers engage in comprehensive discussions and visits encompassing all relevant aspects of the genebank's operations, including those outlined in the Operational Genebank Manual. Additionally, broader issues such as funding stability, management structures and organizational effectiveness are examined. A checklist of key discussion points is available (see Supplemental Material 2).
- The review concludes with a final session dedicated to discussing the observations made by the review team.

Post-review process

- The reviewers prepare a preliminary report summarizing their findings, structured according to the checklist of discussion points.
- The reviewed genebank is given an opportunity to verify the factual accuracy of the report and propose necessary corrections.
- The reviewers finalize the report and provide it to the reviewed genebank for internal use in improvement planning and fundraising efforts.
- The reviewed genebank determines which sections of the report should remain confidential. The public version of the report will reference restricted sections, allowing interested parties to request additional information bilaterally.
- The public report is made available to relevant stakeholders including colleague genebanks and funding agencies (ECPGR, 2025b).

Additional considerations

The review process is designed to be collaborative and founded on mutual trust. The purpose of the review is not to critique individuals but rather to enhance the efficiency and management of the reviewed genebank and exchange knowledge. Consequently, review teams should be kept small, and all activities beyond the core review tasks should be conducted collectively. Furthermore, the proposed cost-sharing arrangement – whereby the host institution covers all local expenses while visiting team members are responsible only for their travel costs to the host country – aims to alleviate financial burdens, particularly for genebanks located in regions with lower cost levels.

Experiences and lessons learned

The concept of Genebank Peer Reviews was formulated in 2019, and since then five cycles of three visits each have been organized in Europe in the frame of Genres Bridge and AGENT projects, involving 12 genebanks (CGN was involved in three cycles, IHAR in two). An overview of the reviews is given in Table 1.

When asked to evaluate their experiences with the Genebank Peer Review, the feedback from the genebanks involved was predominantly positive. The quotes in this paragraph are from this feedback and the evaluation of the first pilot cycle of reviews (van Hintum *et al*, 2019).

Many participants initially needed to familiarize themselves with the concept of Genebank Peer Reviews. As one participating genebank noted, "Although the initial impression was merely that of undergoing an evaluation process to assess the work carried out at the bank, once the process began, the perception shifted to viewing it as an opportunity to receive guidance from personnel with extensive experience in managing germplasm collections."

The process of developing the Operational Genebank Manual was widely regarded as a constructive exercise, offering new insights into the genebank's operations – insights that some genebank managers had not previously considered. However, for genebanks that already had established quality management systems (van Hintum and Wijnker, 2024), the exercise was sometimes perceived as redundant, as it primarily involved restructuring existing quality manuals to fit the Operational Genebank Manual format.

Table 1. The Genebank Peer Reviews performed in the period 2019–2024. For the reports and additional information see https://www.ecpgr.org/aegis/aquas/peer-visits.

Insitute of reviewed genebank	Location visited	Date review visit	Reviewers
Centro de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV)	Valencia, Spain	7-8 February 2019	Theo van Hintum (CGN), Wieslaw Podyma (IHAR-PIB)
Centre for Genetic Resources, the Netherlands (CGN)	Wageningen, The Netherlands	6-8 March 2019	Wieslaw Podyma (IHAR-PIB), María José Díez & José Vicente Valcárcel (COMAV)
National Center for Plant Genetic Resources (IHAR-PIB)	Radzików, Poland	16-17 April 2019	María José Díez & José Vicente Valcárcel (COMAV), Theo van Hintum (CGN)
Crop Research Institute (CRI)	Prague, Czech Republic	12-13 May 2022	Pavol Hauptvogel (RIPP), Ulrike Lohwasser (IPK), Theo van Hintum (CGN)
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)	Gatersleben, Germany	19-20 July 2022	Dagmar Janovská & Ludmila Papoušková (CRI), Pavol Hauptvogel & Iveta Čičová (RIPP)
Research Institute of Plant Production (RIPP)	Piešťany, Slovakia	23-24 August 2022	Dagmar Janovská & Ludmila Papoušková & Vojtěch Holubec (CRI), Ulrike Lohwasser (IPK)
Centro Nacional de Recursos Fitogenéticos (CRF)	Madrid, Spain	7-8 July 2022	Katya Uzundzhalieva & Gergana Desheva (IPGR), Theo van Hintum (CGN)
Centre for Genetic Resources, the Netherlands (CGN)	Wageningen, The Netherlands	19-20 July 2022	Isaura Martin & Luis Guasch (CRF), Katya Uzundzhalieva & Gergana Desheva (IPGR, Bulgaria)
Institute of Plant Genetic Resources 'Konstantin Malkov' IPGR)	Sadovo, Bulgaria	6-7 October 2022	Luis Guasch & Isaura Martín (CRF), Theo van Hintum (CGN)
Nordic Genetic Resource Center (NordGen)	Alnarp, Sweden	29-30 June 2023	John Dickie (MSB), Theo van Hintum (CGN)
Millennium Seed Bank (MSB)	Ardingly, UK	6-7 July 2023	Theo van Hintum (CGN), Lise Lykke Steffensen (NordGen)
Centre for Genetic Resources, the Netherlands (CGN)	Wageningen, The Netherlands	21-22 September 2023	Lise Lykke Steffensen (NordGen), John Dickie & Sharon Balding (MSB)
National Center for Plant Genetic Resources (IHAR-PIB)	Radzików, Poland	21-23 October 2024	Beate Schierscher-Viret (AGROSCOPE), Patrizia Vaccino (CREA-CI)
Research Center for Cereal and Industrial Crops (CREA-CI)	Vercelli, Italy	23-24 September 2024	Maja Boczkowska (IHAR), Beate Schierscher-Viret (AGROSCOPE)
Agroscope Changins (AGROSCOPE)	Nyon, Switzerland	25-26 September 2024	Patrizia Vaccino (CREA-CI), Maja Boczkowska (IHAR)

The cycle of mutual visits was unanimously regarded as a worthwhile investment of both effort and time. "As a host, although the initial sensation was that our genebank was under evaluation, this feeling disappeared as soon as the review started because it was carried out in a friendly atmosphere and we quickly realized that we could take profit of many of the reviewers' suggestions." The feedback provided by reviewing peers was generally perceived as constructive and encouraging. "The opinion of colleagues that are dealing with equivalent responsibilities and problems in another country has given us a better understanding of the strengths and weaknesses of our institution and has served as a starting point for identifying areas of improvement." Moreover, for the reviewing genebanks, the opportunity to closely examine a colleague's genebank proved to be a valuable learning experience. Observing different practices and methodologies served as a source of inspiration, stimulating improvements in approaches and protocols within their own institutions. Furthermore, it strengthened the contacts between genebank managers, or as formulated by one of the participating genebanks: "The two days spent at each genebank not only offered us an opportunity for in-depth discussions on plant genetic resources with a team of experts but also provided an opportunity to strengthen professional relationships with colleagues committed to the same field. We were truly aware of the advantages of being part of a genebank community."

The reports contained between 2 and 21 recommendations by the reviewers. The recommendations addressed very practical issues such as "In order to have unique identifiers for the accessions digital object identifiers (DOIs) should be implemented." or "Consider establishing a lower ceiling to the amount of seeds to be stored of one accession to avoid unnecessary use of space in the -18°C storage room." to policy-oriented recommendations such as "Consider the possibility of introducing handling fees to reduce the requests of hobby growers as the genebank seeds are too expensive to distribute to that category." The reports are available on the peer review website hosted by ECPGR (ECPGR, 2025b).

Assessing the impact of follow-up actions following the review cycles is challenging, as numerous additional factors have also influenced the development of the genebanks involved. Most genebanks utilized the reports to set priorities for their activities and/or to advocate for funding to support specific infrastructural improvements. Additionally, the publicly available Operational Genebank Manuals and review reports (available on the ECPGR/AQUAS website (ECPGR, 2025a)) may have provided valuable insights to other genebanks, potentially inspiring improvements in their operations. At a broader level, these reviews and resulting resources can be expected to have also contributed to increased transparency in genebank practices and methodologies. However, the extent of this influence remains difficult to quantify, although the overall impression is very positive. When asked, one of the genebanks concluded "After several years, participating in the peer review process has led to a substantial improvement in the germplasm bank", a conclusion that is shared by most participating genebanks.

Discussion and conclusions

The conclusions that can be drawn after 15 Genebank Peer Reviews, are largely consistent with those derived from the initial cycle of reviews (van Hintum *et al*, 2019). Firstly, these peer reviews have demonstrated their cost-effectiveness as a means of enhancing the quality of genebank operations. Beyond offering a comprehensive evaluation of various operational aspects, the reviews also play a vital role in motivating and inspiring staff members, thereby promoting continuous improvement.

The reviewers, in general, expressed appreciation for the process, particularly valuing the opportunity to observe and discuss the operations of their colleague genebanks. The social aspect of briefly working alongside international colleagues was also highly regarded.

The functioning of the review teams was generally effective. It became evident that, ideally, one of the genebanks involved should be a well-established institution, capable of serving as an inspiration for others. The review cycle, which involved three genebanks and two or three reviewers for each review, was particularly successful as it allowed for a sufficient level of intimacy, ensuring both confidentiality and transparency. The reviewers were typically sufficiently senior and experienced, enabling them to critically assess their colleagues' work.

The duration of most reviews ranged between one and a half to two days, a timeframe that was deemed short but appropriate. Shorter reviews risked remaining superficial, while longer reviews would have allowed for more in-depth feedback but would also consume more time from the experts involved and thus resources.

The self-assessment process, particularly the preparation of the Operational Genebank Manual prior to the review, emerged as a crucial component in fostering transparency and expanding the hosting experts' own understanding of the procedures within the genebank. In some instances, this process revealed issues that had previously gone unnoticed by the genebank manager. Since the manual does not refer to genebank standards explicitly, the evaluation of the procedures in the context of e.g. the FAO Genebank Standards (FAO, 2014) was one of the aspects of the review.

The Genebank Peer Review approach holds potential for further development into a comprehensive tool not only for enhancing but also for sustaining the quality of genebank operations. To this end, it could be formally integrated into the quality management systems of genebanks, contingent upon its institutionalization within the framework of ECPGR or a comparable overarching body, potentially extending beyond the European context. Such institutionalization would necessitate stable financial support and the establishment of a semi-permanent pool of expert reviewers. Moreover, the systematic follow-up on review recommendations could be embedded as a structural component of genebank quality management, including formalized reporting mechanisms. However, these developments may affect the currently appreciated informal character of the peer review process.

At a broader level, the outcomes of genebank reviews could serve as a valuable resource for informing the prioritization of funding initiatives aimed at strengthening the global system for the conservation of plant genetic resources.

Overall, the Genebank Peer Reviews have had a significant positive impact on the quality of the genebanks involved and have strengthened the connections between genebank experts. They were considered by the reviewers as a cost-effective tool for quality improvement. However, a key challenge remains the reliance on the availability of senior experts, particularly those from well-established institutions. Setting up a pool of experienced genebank experts to 'mentor' the reviews could be a solution.

Supplemental data

Supplemental Material 1: Template for Operational Genebank Manual

Supplemental Material 2: Checklist of Key Discussion Points

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Author contributions

TvH developed the concept of the paper, all other authors commented on the draft and contributed to various extents to the writing.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

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