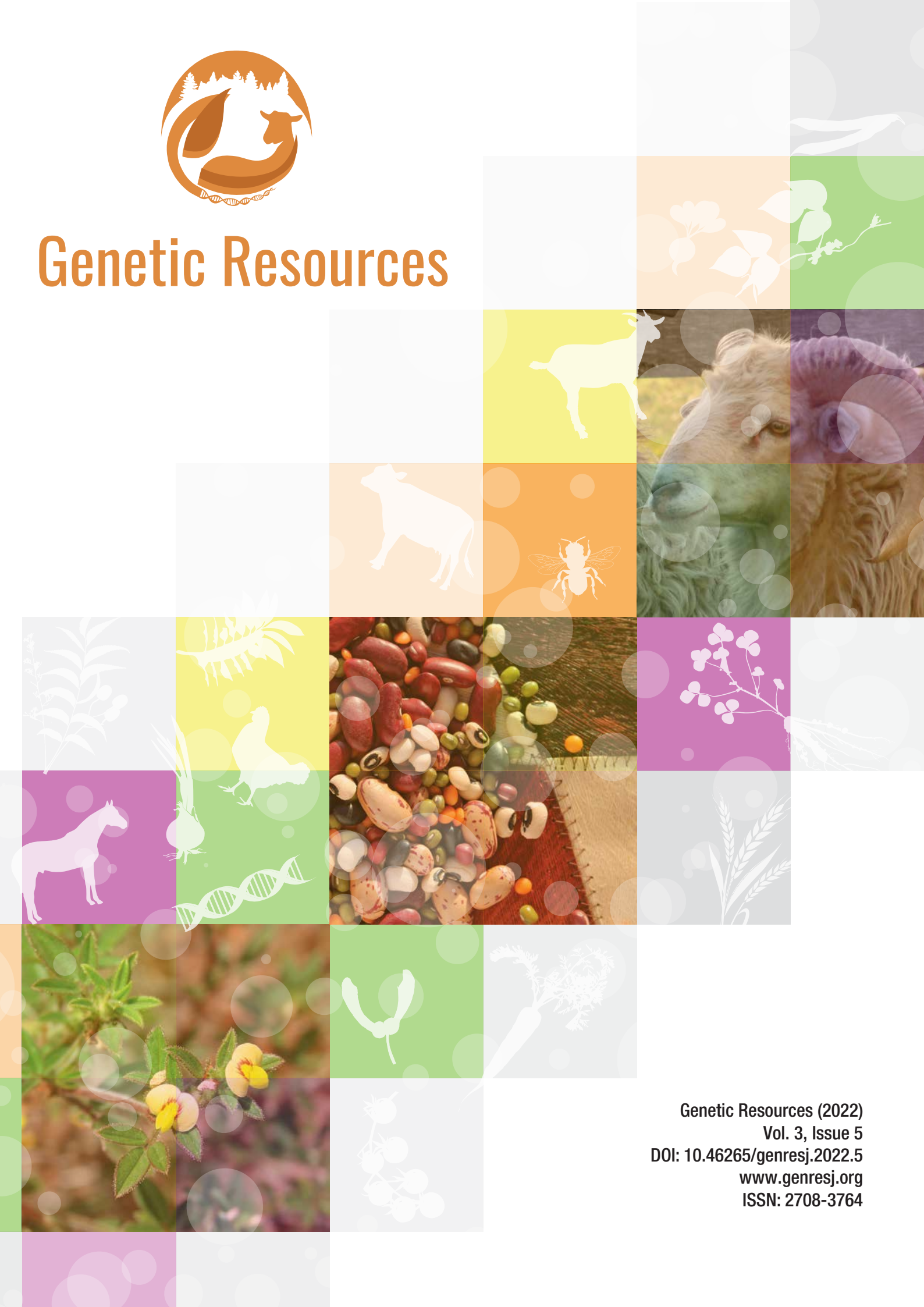




Genetic Resources



Genetic Resources (2022)
Vol. 3, Issue 5
DOI: 10.46265/genresj.2022.5
www.genresj.org
ISSN: 2708-3764

Focus and Scope of *Genetic Resources*

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

Cover photos (from left to right):

Stylosanthes scabra. Credit: João de Deus Medeiros; Diversity of pulses. Credit: Bioversity International/C. Zanzanaini; Creole sheep. Credit: Associação Brasileira de Criadores de Ovinos Crioulos (ABCOC).

© 2022 ECPGR

The designations employed, and the presentation of material in the periodical, and in maps which appear herein, do not imply the expression of any opinion whatsoever on the part of ECPGR concerning the legal status of any country, territory, city or area or its authorities, or concerning the delimitation of its frontiers or boundaries. Similarly, the views expressed are those of the authors and do not necessarily reflect the views of ECPGR.



This journal is supported by the European Cooperative Programme for Plant Genetic Resources (ECPGR) and the European Regional Focal Point for Animal Genetic Resources (ERFP).



This journal has been conceived as part of the [GenRes Bridge](#) project. This project has received seed funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 817580.

Editorial Office:

ECPGR Secretariat
c/o Alliance of Bioversity International and CIAT
Via di San Domenico 1
000153 Rome, Italy

Submissions to www.genresj.org

Editorial Board:

Managing editor:

Sandra Goritschnig (European Cooperative Programme for Plant Genetic Resources, Italy)

Plant Genetic Resources:

Joana Brehm (University of Birmingham, UK)

Igor Loskutov (Vavilov Institute of Plant Genetic Resources, Russia)

Lorenzo Maggioni (European Cooperative Programme for Plant Genetic Resources, Italy)

Nigel Maxted (University of Birmingham, UK)

Jade Phillips (University of Birmingham, UK)

Alvaro Toledo (International Treaty on Plant Genetic Resources for Food and Agriculture, FAO, Italy)

Animal Genetic Resources:

Peer Berg (Norwegian University of Life Sciences, Norway)

Grégoire Leroy (FAO, Italy)

Enrico Sturaro (University of Padova, Italy)

Etienne Verrier (AgroParisTech, France)



Original Articles

The Genetic diversity of Dahanala traditional red rice and molecular markers associated with trichome density on adaxial surfaces

Vidyamali Koodalugodaarachchi, Deepthika S. Kekulandara, Dikkumburage R. Gimhani

Pages 10-23

doi: [10.46265/genresj.AVEO9374](https://doi.org/10.46265/genresj.AVEO9374)

***Stylosanthes scabra*: genetic variability of forage quality traits**

Fabiana Karla de Araújo Américo, Marcelo Ayres Carvalho, Juaci Vitória Malaquias, Allan Kardec Braga Ramos, Gustavo José Braga, Claudio Takao Karia, Carlos Eduardo Lazarini da Fonseca

Pages 24-35

doi: [10.46265/genresj.UPPQ3994](https://doi.org/10.46265/genresj.UPPQ3994)

Phenotypic characterization of Gesha horses in southwestern Ethiopia

Amine Mustefa, Aweke Engdawork, Seble Sinke, Abebe Hailu

Pages 36-50

doi: [10.46265/genresj.KPIL8781](https://doi.org/10.46265/genresj.KPIL8781)

Optimum contribution selection (OCS) analyses prompted successful conservation actions for Faroese Horse population

Anne Kettunen, Signa Kallsoy Joensen, Peer Berg

Pages 59-67

doi: [10.46265/genresj.KKXV5870](https://doi.org/10.46265/genresj.KKXV5870)

Short Communications

A survey on the performance and status of disseminated elite N'Dama cattle breeding bulls at the multiplier tier of an Open Nucleus Breeding Scheme in The Gambia for the period 2011 to 2019

Arss Secka, Lamin Camara, Momodou Jeng, Olawale Olaniyan

Pages 1-9

doi: [10.46265/genresj.SVVL8053](https://doi.org/10.46265/genresj.SVVL8053)

Survey and conservation of crop landraces in northwest Syria

Munzer Al Darvish, Anas Al Kaddour, Akram Bourgol, Yasser Ramazan, Yousef Hallak, Shelagh Kell

Pages 51-58

doi: [10.46265/genresj.USTS1801](https://doi.org/10.46265/genresj.USTS1801)

Conservation status of Creole sheep flocks in Brazil

Gilson Rudinei Pires Moreira

Pages 68-74

doi: [10.46265/genresj.HFHG6814](https://doi.org/10.46265/genresj.HFHG6814)



A survey on the performance and status of disseminated elite N'Dama cattle breeding bulls at the Multiplier tier of an Open Nucleus Breeding Scheme in The Gambia for the period 2011–2019

Arss Secka*, Lamin Camara, Momodou Jeng and Olawale Olaniyan

West Africa Livestock Innovation Centre, PMB 14, Banjul, The Gambia

Abstract: The objectives of this survey conducted in 2020 were to profile Multiplier farmers, assess the performance of disseminated N'Dama breeding bulls at the multiplier cattle herds, and inquire about cattle health and production challenges. Thirty-three farmers living in 33 villages in 5 regions of The Gambia who received 52 elite N'Dama breeding bulls from the West Africa Livestock Innovation Centre (WALIC) Nucleus tier were interviewed.

The results showed that the disseminated bulls' performance is generally satisfactorily as asserted by 28 respondents. Twenty-eight respondents reported that the bulls were healthier, 31 ascribed these bulls with stronger libido, 20 asserted that female offspring from these bulls produced more milk, and 26 claimed that offspring had a faster growth rate. A total of 473 calves have been sired by these bulls thus far. However, only 19 bulls are currently present at the multiplier herds due to the exit of 33 bulls arising from various causes. Cattle production challenges reported by farmers included decreasing grazing lands, rampant bush fires, and inadequate watering points; whilst listed health constraints included inadequate access to veterinary drugs and trypanosomiasis disease prevalence. The multiplication of the disseminated breeding bulls at the Multiplier tier has progressed well in terms of sired offspring, but further transfer to the Commercial Farmer tier is slow. This situation calls for more sensitization of farmers, increased visibility of the breeding programme, and utilization of other reproductive techniques such as artificial insemination to reach more cows for enhanced genetic improvement and productivity.

Keywords: Performance, ONBS, Multiplier tier, N'Dama cattle bulls, The Gambia

Citation: Secka, A., Camara, L., Jeng, M., Olaniyan, O. (2022). A survey on the performance and status of disseminated elite N'Dama cattle breeding bulls at the Multiplier tier of an Open Nucleus Breeding Scheme in The Gambia for the period 2011–2019. *Genetic Resources* 3 (5), 1–9. doi: [10.46265/genresj.SVVL8053](https://doi.org/10.46265/genresj.SVVL8053).

© Copyright 2022 the Authors.

This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

Developing countries like The Gambia have a gross deficit in both beef and milk products as increasing demand far outweighs local production. Due to its tolerance to trypanosomiasis disease, N'Dama cattle is the most widely used breed by farmers in mixed production systems practised in The Gambia, a country which lies within the tsetse infested belt of the African region (Secka *et al*, 2015; Olaniyan *et al*, 2021). N'Dama cattle breed tolerance to trypanosomiasis disease, as

well as their heat tolerance, good draught power and ability to thrive on low-quality feeds, make them highly valuable in such a context. Furthermore, this multi-disease resistant N'Dama cattle has been recommended for low-input traditional African farming systems in areas where trypanosomiasis, ticks and tick-borne diseases are constraints to livestock production (Mattioli *et al*, 1998).

Improving the productivity of N'Dama cattle under low-input production systems through genetic improvement complemented with optimum management is a viable strategy for increasing local meat and milk production using indigenous livestock breeds (Figure 1).

*Corresponding author: Arss Secka (seckaarss@gmail.com)



Figure 1. Selected N'Dama cattle breeding bulls in 2019 for the Multiplier tier. Photo: Arss Secka.

A genetic improvement programme using an Open Nucleus Breeding Scheme (ONBS) involving three tiers was therefore established in 1994 (Bosso, 2007) by the former International Trypanotolerance Centre (ITC), now called West Africa Livestock Innovation Centre (WALIC). The three implicated tiers are Nucleus, Multiplier and Commercial Farmer. The breeding goal of this ONBS is to increase the growth rate and milk yield of indigenous N'Dama cattle without losing their trypanotolerance and other adaptive traits (Jaitner and Dempfle, 1998). Similar breeding schemes for N'Dama cattle genetic improvement are also operational in southern Senegal, Guinea and Mali (Traoré et al, 2017; Camara et al, 2019; Ouédraogo et al, 2021).

This ONBS utilized a young sire scheme to determine the breeding values of young bulls under performance testing from weaning at 12 months of age up to 36 months within a high tsetse challenge area under similar management conditions as practised by community cattle herders. An animal model called Best Linear Unbiased Prediction (BLUP) was used for estimating breeding values for daily weight gain based on monthly weight from 15 to 36 months of age under high tsetse challenge, and the milk yield derived from the first 100 days of lactation was measured on a weekly basis (Bosso et al, 2009).

The young sire scheme was found to be as effective as the half-sib scheme where bulls are selected at the age of 5.5 to 6 years and better than the progeny testing scheme (Dempfle and Jaitner, 2000). These

authors asserted that this scheme is also much simpler to implement, and does not require storing semen or having any waiting bulls.

The Multiplier tier farmers who received elite breeding N'Dama cattle bulls, Djallonké sheep rams, and West African Dwarf goat bucks around the country formed an association called Gambia Indigenous Livestock Multipliers Association (GILMA) in 2002, pulling together most of the farmers at the ONBS Multiplier tier. The main goal of this association was to coordinate multiplication activities of the disseminated breeding males at the Multiplier tier and further dissemination of their male offspring to the Commercial Farmer tier. The association has two main branches, each with its executive committee members, membership and bank account. The branch on the south bank of river Gambia is called GILMA Fulladou, whilst the one on the north bank is called GILMA Saloum. GILMA Associations were supported by ITC and now by WALIC in terms of capacity building, organizational setup and management, technical backup and financial support. After a few years of activity, the GILMAs became dormant in 2007 until ITC commissioned their institutional diagnosis in 2014.

To help revive the GILMAs, ITC/WALIC in collaboration with the West Africa Rural Foundation (WARF), the Regional Project for the Sustainable Management of Endemic Ruminant Livestock in West Africa (PROGEBE) and the Department of Livestock Services, conducted a thorough and exhaustive participatory institutional diag-



Figure 2. Map of The Gambia showing five administrative regions. Source: <https://gisgeography.com/gambia-map/>

nosis (PID) of the association in mid-February 2014. The overall objective of the PID was to develop a pathway for revitalizing the GILMAs into vibrant self-sustaining associations that would effectively carry out their roles and deliver on their responsibilities within the ITC/WALIC breeding programme. Findings showed a lack of clear vision and mission, and limited capacity of the executive committee members in the areas of institutional management, group facilitation, participatory planning, and effective strategic partnership and ownership (Olaniyan *et al*, 2015).

A participatory revitalization plan for the GILMAs was drawn up during the PID exercise. WARF proposed an action plan that involved strategic reflection, capacity building support and coaching/mentoring support to the associations (WARF, 2014). Under the WARF leadership, two workshops were convened in December 2016 and August 2017 with GILMA members for a strategic reflection on and elaboration of the vision, mission and annual work plan; and capacity building on organizational management and group facilitation, communications and information management, resource mobilization, financial management, and rural entrepreneurship respectively. The coaching support to both GILMA Saloum and GILMA Fulladou was carried out in November 2020. During the GILMAs' institutional management strengthening, ITC/WALIC had also conducted four technical capacity strengthening workshops for their membership covering animal breeding and selection, management, animal diseases recognition and control, feeds conservation, pasture production, compost pen construction (ITC, 2017), and hygienic milk collection and processing (ITC, 2018). There has been much improvement in the GILMAs' structural organization and management as well as their technical knowledge and capacity, but the associations have not yet reached the desired level of performance as a livestock breeding association vis-à-vis their vision and mission. The existence of similar breeders' association for the N'Dama cattle breed in Senegal, for Zebus (Azawak and Fulani cattle breeds), Baoulé, and Baoulé x Zebu Crosses in Burkina Faso have been reported (Ouédraogo *et al*, 2021).

The dissemination of elite breeding bulls from the Nucleus tier to the community Multiplier tier resumed in 2011, after a six-year break (2004–2010),

through the intervention of and collaboration with a regional project on sustainable management of endemic ruminant livestock in West Africa, the PROGEBE project (2008–2014), that revived the breeding programme. From 2011 to 2019, a total of 52 elite N'Dama breeding bulls were disseminated to the multiplier farmers at various locations around the country. WALIC make regular biannual monitoring visits to maintain contact with farmers and check on the status of the disseminated breeding bulls. However, gaps exist in the overall profile of the multiplier farmers. The farmers' level of satisfaction or dissatisfaction with the disseminated bulls' general performance over nine years has not been reported. Therefore, this survey aimed at filling the gap by profiling multiplier farmers, assessing the performance of the disseminated elite N'Dama breeding bulls from 2011 to 2019, and inquiring about cattle health and production challenges confronting the farmers.

Materials and Methods

A five-day survey mission was undertaken in April 2020 to interview 40 farmers around the country who received 52 elite N'Dama breeding bulls from ITC/WALIC during the 2011–2019 period, and collect primary data on farmers' profiles, breeding bulls' performance, as well as cattle production and health challenges confronting the farmers. Thirty-three farmers in 33 villages located in five regions of The Gambia (Figure 2) were interviewed during the field mission. The remaining seven target farmers were either not available or had received breeding West Africa Dwarf goat bucks or Djallonke sheep rams whose information are not included in this article.

The target group of this survey was the Multiplier tier of the Open Nucleus Breeding Scheme (ONBS). This second tier of the ONBS consists of farmers whose cattle herds received and used elite N'Dama breeding bulls originating from the on-station WALIC Nucleus tier. As the name implies, the Multiplier tier's role is to produce more outstanding offspring breeding bulls for dissemination to the Commercial Farmer cattle herds around the country.

Two sets of questionnaires were developed for this survey ([Supplemental data](#)). The first questionnaire was a checklist to collect information on farmers' names, village, bull identification number, bull introduction date, bull status, cattle herd size, number of cows in the herd, number of cows mated, number of calves sired, bull exit date and reason for the exit. The second questionnaire was divided into three sections. The first section asked for the beneficiary farmer profile, the second section dealt with account and performance of received animals, and the last section asked for cattle health and production challenges, and their perceived proposed solutions to address these challenges.

The questionnaires were written in English and administered through a face-to-face interview by the authors using common local dialects (Wollof, Mandinka and Fulla) since most of the farmers cannot read or write in English. These dialects are understood by both the interviewers and the interviewees. No intermediary translation was required. Four farmers were partly interviewed through telephone calls when some outstanding information could not be provided by members of the household met on the ground. Telephone calls were also made at times to follow up or verify collected data from the interviewed farmers.

All collected data were organized, sorted, analyzed, and summarized using descriptive statistics into tables and figures showing results of responses to various parameters of interest. The farmers' telephone numbers were used to create a WhatsApp group to serve as a communication platform. The information derived from the accounting of the disseminated bulls provides a record of their status and performance. The responses to the various questions were analyzed to deduce the performance of the bulls, the perceived cattle health and production challenges, and the farmers' proposed solutions to remedy stated challenges.

Results

Profile of the Multiplier tier farmers

The highest number of villages and farmers implicated in this Multiplier tier were found in the districts of Upper Fulladou West, Kiang West, and Niamina East. The age of the interviewed farmers ranged from 43 to 80 years. Out of the 33 farmers, only 2 were female, 3 had western tertiary education (2 Masters and 1 Doctorate holders), and 25 had access to WhatsApp either directly on their phones or a family member's living in the same compound ([Table 1](#)).

Status of disseminated elite N'Dama breeding bulls

A total of 52 elite N'Dama breeding bulls selected from the nucleus herd were disseminated to 35 cattle herds in the 33 villages distributed in 11 districts and 5 regions around The Gambia from 2011 to 2019 ([Table 2](#)). By April 2020, only 19 bulls were present in the multiplier cattle herds around the country ([Supplemental Table 1](#)).

Thirty-three bulls have exited from the community cattle herds due to various reasons such as culling (27%), sales (15%), death (9%), accident (9%), disease (6%), low libido (6%), loss (6%), aggressiveness (6%), and slaughter (3%) ([Supplemental Table 1](#)). Bulls are usually culled after a maximum service period of five years in the same cattle herd as the male breeder. Culling reduced the chances of the bulls mating their daughters thus lowering the risk of inbreeding. Four hundred and seventy-three calves were reported to have been sired by the disseminated bulls from 2011 to 2019 ([Table 2](#)).

Performance of disseminated breeding bulls

Results of the questionnaire survey on the performance of breeding animals aggregated at national level are shown in [Figure 3](#), which provides the results of the questionnaire survey on the performance of breeding animals aggregated at national level.

The health performance parameter measured the resilience of the disseminated bulls to endemic diseases versus other bulls from different sources. Twenty-eight of the 33 respondents asserted that these bulls performed better health-wise than other bulls. The breeding performance parameter compared the libido and fertility of the disseminated bulls with others from different sources. Thirty-one of the 33 respondents affirmed that these bulls had stronger libido and fertility than other bulls. Similarly, the growth performance and milk production of disseminated bulls' offspring were reported faster and higher, respectively, than offspring from other sources. Farmers who received disseminated breeding bulls were satisfied with the general performance of the bulls with their offspring as asserted by 28 respondents compared to 5 respondents that expressed dissatisfaction.

However, most of the farmers had not disseminated mature offspring breeding bulls to Commercial Farmers' cattle herds for breeding purposes. Eight farmers had received bulls less than two years before the survey and had either not seen offspring or female offspring had not started lactating, therefore not responding to questions on offspring growth performance and milk production.

Challenges confronting producers at the Multiplier tier

Data collected from the interviewed farmers focusing on challenges and proposed solutions are presented in [Table 3](#). Most of the health and production challenges highlighted include trypanosomiasis, low access to veterinary drugs and services, inadequate feed and water, bush fires and reduced grazing fields. These challenges could limit the performance of the disseminated animals at the multiplier cattle herds. Farmers' proposed solutions to addressing these challenges include moving towards intensive management of fewer animals for market-oriented enterprise; improving access to veterinary drugs and services; and general public sensitization to reduce bush fire incidents.

Table 1. Profile of the 33 interviewed Multiplier tier farmers

Region	District	No. of villages	No. of farmers interviewed	No. of western-educated farmers	No. accessing WhatsApp	Farmer's gender		Age range (years)
						M	F	
West Coast	Kombo East	1	1	1	1	1	0	67
Lower River	Kiang West	6	6	1	5	5	1	43–70
North Bank	Upper Baddibu	2	3	0	1	3	0	46–56
Central River	Upper Saloum	1	1	0	0	0	1	70
	Nianija	4	4	0	4	4	0	55–80
	Niani	2	2	0	2	2	0	47–59
	Upper Fulladou west	8	6	0	4	6	0	45–70
	Lower Fulladou west	1	1	0	1	1	0	70
	Niamina East	5	5	0	5	5	0	43–66
Upper River	Niamina West	1	1	0	0	1	0	60
	Sandu	2	3	1	2	3	0	54–60
Total	11	33	33	3	25	31	2	43–80

Table 2. Account of disseminated bulls at the Multiplier tier. Additional details are provided in [Supplemental Table 1](#).

Regions	Districts	No. of villages	No. of cattle herds implicated	No. of bulls supplied (2011–2019)	No. of bulls present in 2020	No. of calves sired (2011–2019)
West Coast	Kombo East	1	1	2	1	24
Lower River	Kiang West	6	6	6	2	64
North Bank	Upper Baddibu	2	3	4	2	105
Central River	Upper Saloum	1	1	1	1	0
	Nianija	4	4	4	0	145
	Niani	2	3	3	3	24
	Upper Fulladou West	8	7	16	4	38
	Lower Fulladou West	1	1	2	2	0
	Niamina East	5	5	9	2	25
	Niamina West	1	1	1	0	28
Upper River	Sandu	2	3	4	2	20
Total	11	33	35	52	19	473

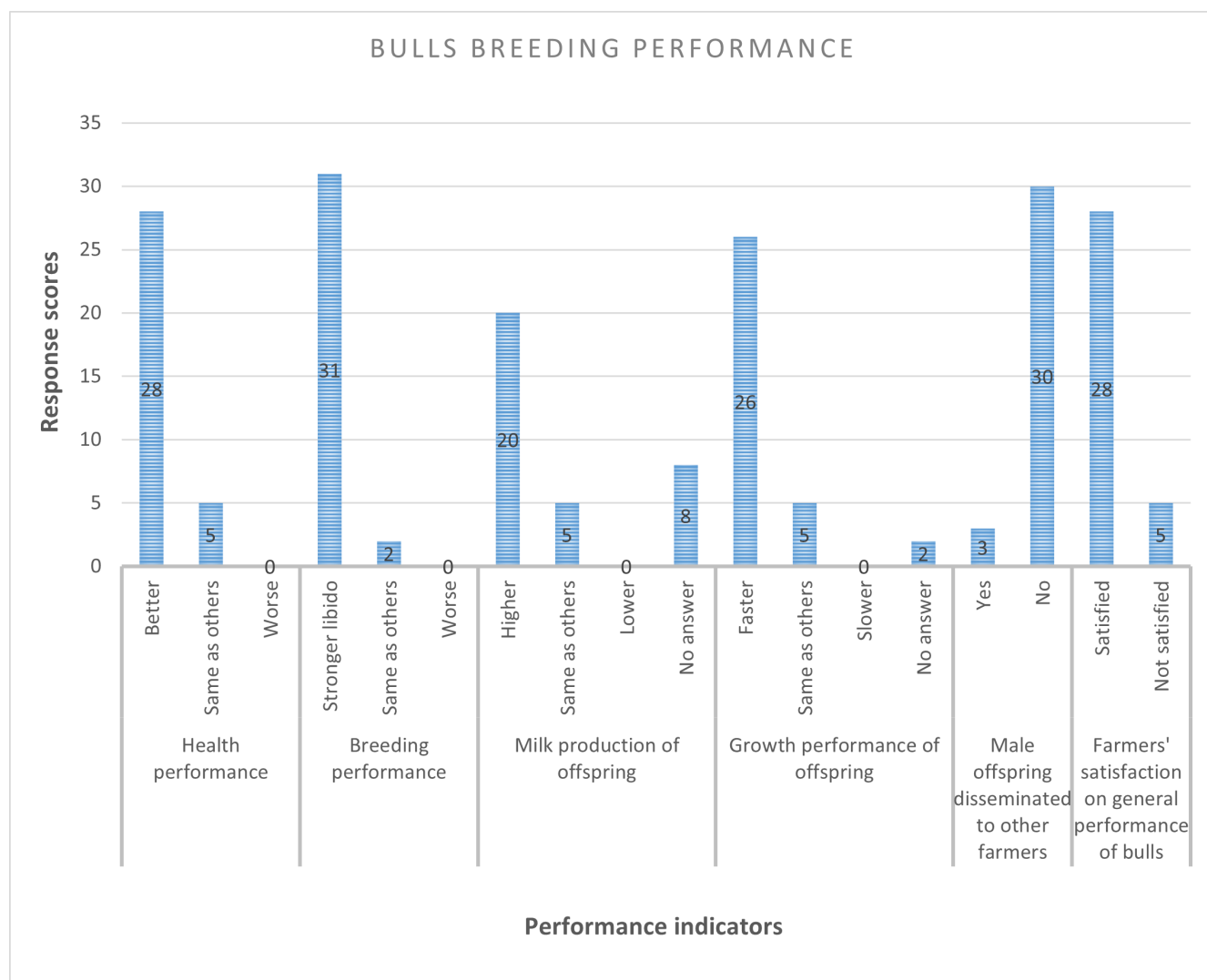


Figure 3. Responses of 33 Multiplier tier farmers to bulls breeding performance indicators

Table 3. Challenges confronting livestock producers and proposed solutions

Region	No. of districts	No. of farmers interviewed	Health challenges	Production challenges	Marketing challenges	Proposed solutions
West Coast	1	1	None	Lack of sufficient land for free grazing of cattle	None	Introduce techniques of intensive production using exotic breeds to increase milk and meat production
Lower River	2	5	Access to veterinary drugs	Bush fires causing feed shortages during the dry season	None	Improve access to veterinary drugs and services. Sensitization activities to reduce bushfires in the dry seasons especially in Kiang West District
North Bank	1	3	Trypanosomiasis infections in dry seasons	Insufficient clean drinking water for the cattle during the dry season	None	Improve access to veterinary drugs and provision of drinking points for cattle
Central River	6	21	Access to veterinary drugs	Insufficient grazing lands	None	Improved access to veterinary services and drugs.
Upper River	1	3	None	Lack of clean drinking water	None	Provide cattle drinking points

Discussion

Multiplier tier farmers' profile

Among the 33 farmers who were interviewed, 30 were illiterate in the English language, whilst three are highly educated professionals in the western form of education system. The presence of only 2 women out of 33 interviewed farmers show that male farmers dominate the ONBS cattle Multiplier tier around the country. This observation follows the national pattern of males dominating the ownership of cattle production and management in The Gambia. The age of the interviewed multiplier farmers ranging from 43 to 80 years is indicative of the ages of the household heads. In most instances, the head of the family owns the disseminated elite breeding bulls. However, the daily management of the cattle herds rests on the shoulders of younger family members or contracted herdsmen.

Account of disseminated bulls

The districts of Upper Fulladou West, Niamina East, Kiang West, and Nianija received most of the disseminated bulls. This observed distribution pattern is explained by the fact that the first three districts have been participating in many ITC/WALIC activities, particularly the genetic improvement programme. Moreover, the districts of Kiang West, Niamina East and Nianija were also intervention sites of the Regional project for the Sustainable Management of Endemic Ruminant Livestock in West Africa (PROGEBE) in 2008–2014, which had a component of supplying improved breeding bulls from ITC/WALIC to their contact cattle farmers. Out of the 52 disseminated bulls, as of April 2020, only 19 were present in Multiplier tier cattle herds due to various reasons, as shown in [Table 2](#).

Performance of disseminated breeding bulls

The findings on the breeding performance of disseminated bulls at the Multiplier tier showed that the breeding programme was attaining its target breeding goal of increased growth rate and milk offtake without compromising the tolerance to endemic diseases like trypanosomiasis. Both the health and breeding performance of the disseminated bulls at the Multiplier tier got high scores from the respondents. Therefore, it appears that the high genetic potential for higher growth rate and milk offtake contained in these bulls had been transferred to their offspring.

This survey's findings corroborate a survey conducted in 2003, which assessed the adoption and impact of the ITC/WALIC genetic improvement programme at the Multiplier tier ([Agyemang, 2003](#)). Findings from the 2003 survey showed that participating villages, associations and households were pleased with the benefits from the improvement programme and disseminated breeding bulls performed well under village conditions. All participating respondents believed that the use of improved N'Dama bulls would improve their livelihoods. Although the genetic gains at the

Multiplier tier had not been calculated during the 2003 survey, [Mattioli *et al* \(1998\)](#) found that from 1994 to 2004 the estimated average breeding values for weight at 36 months ranged between 0 to 6.32kg at the Nucleus tier. They further asserted that weight at 36 months showed the highest genetic gain with a response of 0.40kg per year. Hence, it is highly probable that the bulls disseminated to the multiplier farmers from 2011 to 2019 may have had similar or higher genetic gains for weight at 36 months.

Siring many calves at the Multiplier tier cattle herds is one of the ultimate aims of disseminating improved breeding N'Dama bulls from the Nucleus tier. Disseminated bulls during this period have sired a total of 473 healthy and strong calves. Further dissemination of bulls' male offspring from the Multiplier tier to the Commercial Farmer tier was found to be limited – only two offspring bulls in 2018 and one in 2019. This challenge has been recognized for some years now and efforts have been taken to remedy the situation. Institutional diagnostics of GILMAs in 2014 showed that there are many organizational, management, capacity and financial limitations affecting the association. Activities for strengthening the GILMAs have been implemented and hopefully, this situation of low transmission of breeding bulls from the Multiplier tier to the Commercial Farmer tier will improve in the near future. It also appears that many livestock farmers are not aware of the WALIC breeding programme. This calls for more sensitization of farmers and increased visibility of the programme. Artificial insemination is also a faster reproductive tool where stored semen from bulls could be used in many cows for a longer duration than live bulls.

Challenges confronting livestock producers

The expression of disseminated bulls' genetic values could be affected by environmental factors such as feed, management, water, changing climate and disease-causing pathogens. Various challenges on health, production and marketing were assessed during this survey. Although there seem to be no marketing challenges, respondents asserted some challenges under health and production domains. Access to veterinary drugs was reported as a limitation in both Central River and Lower River Regions. This could be explained by the fact that private veterinary drug outlets operating in these regions are located mainly in towns and big villages, whilst these farmers are mostly residing in small villages. However, they could still travel to the veterinary outlets or call the public livestock officers to attend to their animals' health needs.

Trypanosomiasis was mentioned as a challenge in North Bank Region particularly during the dry season when the animals are nutritionally stressed. N'Dama cattle are trypanotolerant, hence would still survive and produce in trypanosomiasis prevalent areas ([Mattioli *et al*, 1998](#)).

The major highlighted cattle production challenges were inadequate grazing lands, the occurrence of bush fires ravaging pastures and rangelands, and insufficient cattle drinking points. These challenges are very serious factors that could retard the progression of increased livestock productivity at both Multiplier and Commercial Farmer tiers. Adequate good-quality feed and water are essential for growth, maintenance and production. Grazing lands are dwindling, resulting from increasing human population pressure and associated competing agro-industrial and residential activities. Conflicts may arise between pastoralists and farmers during cropping seasons as access routes to the grazing areas are often blocked. This situation leads to the transhumance of cattle to other regions in The Gambia or the surrounding Republic of Senegal. Cattle drinking points are limited and these negative impacts are felt during the long dry season.

Many solutions were proposed by the respondents to solve these challenges. They include the use of exotic cattle breeds under intensive management for increased meat and milk production; community sensitizations to stop the harmful practice of bush fires; government support for the provision of cattle watering points; and improving access to veterinary services and drugs. Embracing the proposed solutions requires capital investment from farmers, the private and public sectors, and non-governmental organizations (NGOs). There are some initiatives taken up by the government and development partners to address these challenges and are registering some significant progress.

Conclusion

Although the survey has used mainly qualitative data obtained from farmers' perceptions and recollections and not backed by longitudinal quantitative production data, it has provided some important insights about Multiplier tier farmers, the performance of disseminated bulls, and constraints limiting cattle production and productivity.

The survey has profiled the farmers involved in the ongoing breeding programme and the general performance of the disseminated bulls at the Multiplier tier. Farmers – more than 40 years old, predominantly male and very few with higher education – generally practise subsistence farming and extensive management of N'Dama cattle herds combined with the cultivation of crops. Farmers are generally satisfied with the health and breeding performances of the disseminated breeding bulls at the Multiplier tier. They have asserted that both the growth rate and milk outputs of these bulls' offspring are higher than those from other bulls. Only three offspring bulls were disseminated from the Multiplier tier farmers to the Commercial Farmer tier cattle herds as breeders. This figure is very low and therefore the following actions are proposed: 1) more sensitization of farmers, 2) increased visibility of the breeding programme, and 3) use of other dissemination tools such as artificial insemination that could reach

more cows for enhanced genetic improvement and productivity.

Major health challenges mentioned by the farmers include trypanosomiasis and inadequate access to veterinary services and drugs. On production challenges, issues include insufficient grazing lands, frequent bush fires ravaging large amounts of fodder, and insufficient livestock watering points during the long dry season. Many initiatives are taken by individual farmers, communities, private and public sector-led projects to address prevailing livestock production and health challenges. The Gambia Indigenous Multipliers Association (GILMA) has been found weak, and activities were conducted to revamp this association to facilitate and sustain the transfer of improved genetic gains across the three-tier open nucleus breeding scheme.

Acknowledgements

The authors are grateful to the Government of The Gambia for providing financial support in the form of monthly subventions that keep the WALIC staff personnel on board and facilitated travel costs for the collection of data during the survey period.

Supplemental Data

Questionnaires used in farmer's survey:

[Questionnaire 1](#): Check list for disseminated bulls

[Questionnaire 2](#): Performance of elite N'Dama breeding bulls at the Multiplier tier

[Supplemental Table](#). Checklist data on account of disseminated bulls

[Translated Abstract in French](#)

Author contributions

Arss Secka coordinated the survey, took part in the collection, collation and analysis of data from farmers, and drafted the article manuscript. Lamin Camara provided inputs into the manuscript and participated in the collection, collation and analysis of data from farmers. Momodou Jeng provided inputs into the manuscript and participated in the data collection from farmers. Olawale Olaniyan provided inputs into the manuscript.

Conflict of interest statement

The authors declared that there is no conflict of interest.

References

- Agyemang, K. (2003). Improved Performance in Trypanotolerant Livestock. Final Project Report of BMZ/GTZ funded project.
- Bosso, N. (2007). The N'Dama cattle genetic improvement programme: A review. *Animal Genetic Resources Information* 40, 65–69. doi: <https://doi.org/10.1017/S1014233900002200>

- Bosso, N. A., Van Der Waaij, E. H., Kahi, A. K., and Van Arendonk, J. A. M. (2009). Genetic analyses of N'Dama cattle breed selection schemes. *Livestock Research for Rural Development* 21(8). url: <http://www.lrrd.org/lrrd21/8/bosso21135.htm>.
- Camara, Y., Moula, N., Sow, F., Sissokho, M. M., and Antoine-Moussiaux, N. (2019). Analysing innovations among cattle smallholders to evaluate the adequacy of breeding programs. *Animal* 13(2), 417–426. doi: <https://doi.org/10.1017/S1751731118001544>
- Dempfle, L. and Jaitner, J. (2000). Case Study about the N'Dama breeding programme at the International Trypanotolerance Centre (ITC) in The Gambia. In Galal, S., Boyazoglu, J., and Hammond, K., Developing Breeding Strategies for Lower Input Animal Production Environments. Proc. of an International Workshop, Bella (Italy), 22-25 September 1999, volume 3 of *ICAR Technical Series*, ICAR, Rome, Italy, 347-354.
- ITC (2017). ITC Annual Report 2017 and Outlook 2018. International Trypanotolerance Centre, Banjul, The Gambia.
- ITC (2018). ITC Annual Report 2018 and Outlook 2019. International Trypanotolerance Centre, Banjul, The Gambia.
- Jaitner, J. and Dempfle, L. (1998). A breeding scheme for the indigenous N'Dama cattle. In Proceedings of the 49th annual meeting of the EAAP, 24–27 August 1998, Warsaw, Poland, 42p.
- Mattioli, R. C., Jaitner, J., Clifford, D. J., Pandey, V. S., and Verhulst, A. (1998). Trypanosome infections and tick infestations: susceptibility in N'Dama, Gobra zebu and Gobra x N'Dama crossbred cattle exposed to natural challenge and maintained under high and low surveillance of trypanosome infections. *Acta Tropica* (1), 57–71. doi: [https://doi.org/10.1016/S0001-706X\(98\)00051-5](https://doi.org/10.1016/S0001-706X(98)00051-5)
- Olaniyan, O. F., Fall-Diop, N. N., B, F., Secka, A., Smith, O. B., and Kebbeh, M. (2015). Assessment of institutional capacities of the Gambia Indigenous Livestock Multipliers' Association. *Animal Genetic Resources* 57, 119–126. doi: <https://doi.org/10.1017/S207863361500020X>
- Olaniyan, O. F., Kaya, I., and Secka, A. (2021). Trypanosomosis prevalence in natural field-based infection: insights into systematic review and meta-analysis of studies from 1980 to 2018 on The Gambian ruminants with special emphasis on cattle. *Tropical Animal Health and Production* (25), 53–53. doi: <https://doi.org/10.1007/s11250-020-02461-w>
- Ouédraogo, D., Soudré, A., Yougbaré, B., Ouédraogo-Koné, S., Zoma-Traoré, B., Khayatzaadeh, N., Traoré, A., Sanou, M., Mészáros, G., Burger, P. A., Mwai, O. A., Wurzing, M., and Sölkner, J. (2021). Genetic Improvement of Local Cattle Breeds in West Africa: A review of Breeding Programs. *Sustainability* 13(4), 2125. doi: <https://doi.org/10.3390/su13042125>
- Secka, A., Gibba, P., Ceesay, A., Bojang, M., Jarju, A., and Gaye, M. (2015). Effects of changing climate and vegetation on trypanosomosis burden in The Gambia. *Bulletin of Animal Health and Production* 63(3), 335–367. url: <http://repository.au-ibar.org/handle/123456789/476>.
- Traoré, S. A., Markemann, A., Reiber, C., Piepho, H. P., and Valle Zárate, A. (2017). Production objectives, trait and breed preferences of farmers keeping N'Dama, Fulani Zebu and crossbred cattle and implications for breeding programs. *Animal* 11(4), 687–695. doi: <https://doi.org/10.1017/S1751731116002196>
- WARF (2014). Participatory Institutional Diagnosis of Gambia Indigenous Multipliers Association. West Africa Rural Foundation, Senegal and International Trypanotolerance Centre, The Gambia.



Genetic diversity of *Oryza sativa* 'Dahanala' traditional red rice and molecular markers associated with trichome density on adaxial surfaces

Vidyamali Koodalugodaarachchi^a, Deepthika S Kekulandara^{*,b} and Dikkumburage R Gimhani^a

^a Department of Biotechnology, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Gonawila (NWP), 60170, Makandura, Sri Lanka

^b Plant Genetic Resources Centre, 20400, Gannoruwa, Peradeniya, Sri Lanka

Abstract: *Oryza sativa* 'Dahanala' is a traditional red rice variety acknowledged for its thrips resistance. This study focused on the genetic diversity assessment of Dahanala accessions in Sri Lanka. Twenty-six Dahanala accessions conserved at the genebank of the Plant Genetic Resources Centre and six accessions from the Rice Research and Development Institute, Batalagoda, were analyzed using seven seed morphology characters followed by molecular characterization with 31 simple sequence repeat markers, showing a significant genetic variation of the accessions. Accessions 003924/003327 and 0010160/006165 were reported as two potential pairs of duplicates. The polymorphic information content values varied between 0 (RM255) and 0.697 (RM412). Genetic distance ranged within 0.0 and 0.94 revealing considerable genetic variance. Sixteen closely related accessions were selected as a representative set of Dahanala, including accessions 003924, 003327, 006376, 010160, 006165, 006378, 004968, 003304, 006739, 005386, 004507, 003149, 003131, 627, 626 and 629. To assess the variation of trichome phenotypes among Dahanala accessions, leaf trichome density as well as RM277 and RM279 markers, which carry a putative relationship with thrips resistance, were used as morphological and molecular markers, respectively. Results revealed a variation of trichome density from 6.7 to 30.83 trichomes/mm². According to the molecular marker analysis, both markers revealed polymorphism in thrips-resistant Dahanala accessions and susceptible *Oryza sativa* 'Sudur Samba' accessions, but no clear linkage between the markers and trichome phenotypes within the selected Dahanala accessions could be found. Further studies are needed to dissect the relationship between trichome phenotype and thrips resistance in red rice.

Keywords: Dahanala, Genetic diversity, Rice, Simple sequence repeat markers, Trichome density

Citation: Koodalugodaarachchi, V., Kekulandara, D. S., Gimhani, D. R. (2022). Genetic diversity of *Oryza sativa* 'Dahanala' traditional red rice and molecular markers associated with trichome density on adaxial surfaces. *Genetic Resources* 3 (5), 10–23. doi: [10.46265/genresj.AVEO9374](https://doi.org/10.46265/genresj.AVEO9374).

© Copyright 2022 the Authors.

This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

As the oldest domesticated crop since more than 10,000 years, rice (*Oryza sativa*) has become central to the lives of billions of people around the world and is rated as the world's second most important cereal crop following only corn (Gramene; Shahbandeh, 2022). In 2019, with a production volume of over 209Mt, China

was ranked as the world's largest rice producer followed by India and Indonesia. Though the total world rice production reached nearly 496Mt in 2020, the supply is not sufficient to fulfil the rising demand of an increasing global population and decreasing cultivable land (Shahbandeh, 2022). Most of the lands used for rice cultivation in those countries, including Sri Lanka, are occupied with new improved varieties (NIV), while traditional varieties are cultivated to a minor extent. Despite its lower productivity, emerging knowledge of traditional rice health benefits has contributed to

*Corresponding author: Deepthika S Kekulandara (deepthikasaman@gmail.com)

increasing its market value (Wickramasinghe and Noda, 2008). Beneficial qualities include antioxidant and indigenous medicinal properties, good nutritional values and good quality attributes of the grains (Suriyagoda et al, 2011). Most prominently, resistance towards biotic and abiotic stresses has been shown for traditional varieties whereas many of the NIV remain susceptible to those stress conditions (Wickramasinghe et al, 2007).

Oryza sativa 'Dahanala' is a traditional red rice variety conserved at the Plant Genetic Resources Centre (PGRC) genebank, Gannoruwa, Sri Lanka, and is acknowledged for its indigenous medicinal value. Furthermore, the characterization of national rice germplasm has been able to identify traditional rice varieties such as Dahanala and Wanni Dahanala as donors of thrips resistance (Kudagamage, 1977; Nugaliyadde and Heinrichs, 1984). The thrips resistance of some traditional rice varieties may be attributed to a higher density of non-glandular trichomes on the adaxial surface of the leaves, which contributes to the plant's antixenosis ability by preventing thrips from landing on the plant surface and inhibiting their movements and feeding (Panda and Khush, 1995).

Simple sequence repeats (SSR) are defined as a small sequence of DNA that contains repeat motifs ranging in size between one and six base pairs. Unlike other DNA markers, such as restriction fragment length polymorphism (RFLP), random amplification of polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP), microsatellite SSR markers have been preferred by scientists due to their ability to represent the polymorphic behaviour of an individual by producing comparatively higher informative bands along with co-dominant nature and reproducibility of the results (Korzun, 2002).

A considerable collection of highly reproducible microsatellite markers (SSR), dispersed throughout 12 chromosomes of the rice genome, is available (McCouch et al, 2001). Since their development in 1985, microsatellites have been used for characterizing populations, diagnosing certain genotypic characters through DNA fingerprinting and screening different types of individuals (Jeffreys et al, 1985).

Within a breeding programme to develop thrips-resistant improved rice cultivars, microsatellites markers linked to thrips resistance in rice were identified using bulk segregant analysis of F_2 progeny derived from a cross between thrips resistant Dahanala and thrips susceptible Suduru Samba rice varieties (Gimhani, 2010). According to the findings, a putative association of thrips resistance with RM279 and RM277 markers and evidences for a possible major QTL responsible for thrips resistance close to the RM279 marker locus were discovered (Gimhani, 2010). Moreover, a significant association between markers RM277 and RM279 and higher trichome density on the adaxial surface of the leaf was also found.

In Sri Lanka, a total of 32 Dahanala accessions are conserved in genebanks, including 26 accessions

at the PGRC genebank, Gannoruwa, and 6 accessions at the Rice Research Development Institute (RRDI), Batalagoda. As these accessions are conserved by the vernacular names given by farmers (Wanni Dahanala, Dhana Hala, Dhanala, Danahala and Dahanala), the possibility of conserving duplicates of the same cultivar under different names or, conversely, different cultivars under the same name, is high.

The objective of the present study was to analyze the genetic diversity of 32 accessions of the Dahanala traditional rice variety conserved in Sri Lanka, using both morphological and molecular parameters. Furthermore, the study scope was targeted on the evaluation of trichome densities and the presence of RM277 and RM279 marker loci in Dahanala accessions as putative correlation with thrips resistance, thus providing information on a potentially valuable breeding resource.

Materials and methods

Plant materials

Thirty-two Dahanala accessions were included in this study, listed in Table 1. Seeds of 26 accessions were obtained from the PGRC genebank, Gannoruwa, Sri Lanka, and six accessions from RRDI, Batalagoda, Sri Lanka. Accession 006739 is the oldest Dahanala line available in Sri Lankan genebanks. The advanced breeding line *Oryza sativa* Bg 360 was used as reference variety during the analysis.

Morphological seed characterization

Five replicated seeds of each Dahanala accession were evaluated with seven qualitative and quantitative characters (i.e. seed shape, colour and pubescence of lemma and palea, seed coat colour, sterile lemma colour, grain length and grain width), using a modified rice seed evaluation list based on the International Plant Genetic Resources Institute rice descriptors (Bioversity International, IRRI and WARDA, 2007). See Supplemental Data 1 for a detailed description of seed morphological characters.

Molecular characterization

DNA extraction

Genomic DNA of each accession was extracted from 3-week-old immature leaves using a modified cetyltrimethylammonium bromide (CTAB) method (Murray and Thompson, 1980) with some optimizations at PGRC, Gannoruwa.

About 20–30 seeds per accession were cleaned well and allowed to soak in distilled water in Petri dishes for 24 hours to enhance germination. The soaked seeds were then transferred onto wet tissues inside the labelled Petri dishes for germination. Distilled water and Albert's solution (1g/450ml) were added from time to time to maintain the moisture and nutrient level required by the seedlings. Seedlings were inspected for any fungal contamination and infected

Table 1. Details of plant material used in the study. PGRC, Plant Genetic Resources Centre, Gannoruwa, Sri Lanka; RRDI, Rice Research Development Institute, Batalegoda, Sri Lanka; CRBS, Central Rice Breeding Station (presently named RRDI), Batalagoda, Sri Lanka; CARI, Central Agricultural Research Institute, Sri Lanka; IRRI, International Rice Research Institute, Los Baños, the Philippines.

No.	Accession Number	Conserved Location	Cultivar Name	Origin
1	002049	PGRC Genebank	Dahanala	CARI
2	002050	PGRC Genebank	Dahanala	CARI
3	002053	PGRC Genebank	Dahanala	Unknown
4	003131	PGRC Genebank	Dahanala 2014	Unknown
5	003149	PGRC Genebank	Dahanala 37 YM 2014	Unknown
6	003386	PGRC Genebank	Dahanala	Unknown
7	003540	PGRC Genebank	Dahanala	Unknown
8	003917	PGRC Genebank	Dahanala	Unknown
9	003924	PGRC Genebank	Dahanala	Unknown
10	003971	PGRC Genebank	Dahanala	Unknown
11	004030	PGRC Genebank	Dahanala	Unknown
12	004968	PGRC Genebank	Dahanala	Unknown
13	006165	PGRC Genebank	Dahanala	Unknown
14	006376	PGRC Genebank	Dahanala	Unknown
15	006377	PGRC Genebank	Dahanala	Unknown
16	006378	PGRC Genebank	Dahanala	Unknown
17	006739	PGRC Genebank	Dahanala	IRRI
18	010160	PGRC Genebank	Dahanala	Unknown
19	014122	PGRC Genebank	Dahanala	Unknown
20	015533	PGRC Genebank	Dahanala	Unknown
21	006357	PGRC Genebank	Wanni Dahanala	Unknown
22	006358	PGRC Genebank	Wanni Dahanala	Unknown
23	003304	PGRC Genebank	Dahanala	Unknown
24	003327	PGRC Genebank	Dahanala	Unknown
25	004507	PGRC Genebank	Dhana Hala	Unknown
26	005386	PGRC Genebank	Dhanala	CRBS
27	592	RRDI	Wanni Dahanala	CRBS
28	626	RRDI	Dahanala	CRBS
29	627	RRDI	Dahanala	CRBS
30	629	RRDI	Dahanala	CRBS
31	1214	RRDI	Dahanala	CRBS
32	1246	RRDI	Dahanala	CRBS
33	Advanced breeding line	RRDI	Bg 360	
34	003333	PGRC Genebank	Suduru Samba	Unknown
35		RRDI	Suduru Samba	Unknown

ones were discarded. About one week after germination, seedlings were transferred to pots filled with mud at the plant house. Two weeks after transplanting, juvenile immature leaves were harvested for DNA extraction. Two grams of fresh leaf samples were ground well with liquid nitrogen using mortar and pestle until a fine powder was formed. The powdered sample was transferred into Oak Ridge centrifuge tubes with 4ml of preheated (65°C) 2% CTAB extraction buffer. Subsequently, 1.2µl of 0.2% β-mercaptoethanol was added to each tube. The tubes were incubated at 65°C for 30 minutes in a water bath. After that, an equal amount (4ml) of chloroform:isoamyl alcohol (24:1) was

added to each tube. The tubes were slowly shaken for 10 minutes in a shaker.

All the tubes were centrifuged at 8,000rpm for 15 minutes. The supernatant was transferred into a new tube without disturbing the interface. Then an equal volume of chloroform:isoamyl alcohol (24:1) was added to each tube. After that, the tubes were centrifuged at 8,000rpm for 15 minutes. The supernatant was transferred into a new vial and an equal volume of chloroform:isoamyl alcohol (24:1) was added again. After the repeated centrifugation step (8,000rpm) pelleted DNA was spooled out, transferred to a centrifuge tube and washed by adding 70% ethanol followed by centrifugation at 10,000rpm for 5 minutes.

DNA pellets were air-dried for 3–4 hours and stored in TE buffer at -20°C. DNA integrity checking and quantification were carried out using 0.8% Agarose gel electrophoresis.

Microsatellite marker characterization

Thirty-one microsatellite markers dispersed broadly over the 12 rice chromosomes were selected from the published sequence database ([Gramene Microsat](#)). The primer sequence information is provided in [Table 2](#). In addition, markers RM277 and RM279, previously shown to be correlated with trichome density and thrips resistance, were tested on selected accessions.

Primer optimization

Prior to genotyping of each accession, the annealing temperature for each primer was optimized using the temperature gradient method to avoid non-specific PCR amplification. PCR amplification was carried out for all 31 primer pairs using an annealing temperature range between 51°C and 61°C. Amplified PCR products were confirmed by using 1.5% agarose gel electrophoresis in 0.5x TBE buffer at 90V for 45 minutes to 2 hours, based on the product size. The annealing temperature with minimum non-specific products was selected for each primer for PCR amplification ([Table 2](#)).

Molecular assessment of DNA bulks

To reduce costs, DNA samples were bulked for initial genotyping of Dahanala using the selected 31 SSR primers. PCR amplifications were performed using Applied Biosystems 9902 thermal cycler. The initial denaturation step was performed at 94°C for 4 minutes, followed by 35 cycles of denaturation at 95 °C for 1 minute, primer annealing at optimized temperatures (55°C/57°C/59°C/61°C) for 1 minute, extension at 72°C for 2 minutes, and final extension at 72°C for 5 minutes. Amplified PCR products were confirmed by 1.5% agarose gel electrophoresis.

Confirmed amplified PCR products of all 31 SSR primers were resolved using 8% non-denaturing polyacrylamide gel (acrylamide:bisacrylamide ratio of 29:1) electrophoresis (PAGE) using 1x TBE buffer and DNA bands were visualized by the Bio-Rad gel documentation system with the assistance of Quantity One software. Where necessary, the DNA bulks were resolved to separate divergent markers.

Trichome density analysis

A total of 297 rice seedlings were transplanted representing all 32 accessions of Dahanala along with the reference variety (Bg 360) into pots filled with mud at the PGRC plant house. Trichome density was determined 30 days after transplanting, using the third leaves of three randomly selected plants for each accession. The trichome count was acquired by observing the first $\frac{1}{4}$ area of the adaxial surface of the leaf blade from the tip of each sample under a light microscope keeping a paper strip with a $2 \times 3 \text{ mm}^2$ square hole on it. Nine readings per individual were recorded.

The average trichome density (trichome number/ mm^2) per accession was calculated from the mean of three counts per sample ([Wickramasinghe et al, 2007](#)).

Data scoring and analysis

Morphological characterization: Cluster analysis of morphological data was carried out through the average linkage method and Euclidean distance using Minitab version 15 ([Minitab, 1991](#)).

Molecular characterization: Gel images were scored manually by visual observation as presence (1) and absence (0) of every allele for all 31 pairs of SSR markers. DNA bands of expanded bulks were scored individually along with non-expanded bulks. Scored data were analyzed by Power Marker version 3.25 ([Liu and Muse, 2005](#)) calculating major allele frequency, polymorphic information content (PIC) value and Nei's genetic distance ([Nei et al, 1983](#)) across the 32 accessions to identify the prevailing genetic diversity. The phylogenetic tree was constructed based on Nei's genetic distance ([Nei et al, 1983](#)) according to neighbour-joining method using MEGA 6.06 software ([Tamura et al, 2013](#)).

Trichome density analysis: The trichome density distribution of the population was assessed through the Kruskal-Wallis test at 0.05 significance level by using Minitab version 15.

Results

Morphological characterization of 32 Dahanala accessions

Considerable variation in seed morphology was observed among the 32 Dahanala accessions ([Table 3](#), [Figure 1](#)). Grain shapes were either half-spindle shaped (6–7mm) or spindle shaped (7–8mm) whilst vast variation was identified in lemma and palea colour. The majority of accessions had brownish-black lemma and palea colour, only one had brownish-black furrows on straw background (Acc. No. 003971). All seeds had red pericarp and straw-coloured sterile lemma, showing no diversity in these traits. The nature of the pubescence varied between short hairs and hairs on the upper portion. Grain width ranged from 2.858mm to 3.508mm while grain length varied from 9.314mm to 6.946mm.

In a dendrogram based on the seed morphological characters, the 32 accessions clustered into eight groups at 91.18% similarity level showing their potential closer relationship ([Figure 2](#)). Accessions 003971, 002049, 002050 and 014122 clustered independently and distantly denoting morphological deviations from the rest of the accessions.

Molecular assessment of Dahanala accessions using SSR microsatellite markers

To reduce costs, the 32 DNA samples were pooled into eight bulks at 91.18% similarity level, based on the dendrogram resulting from seed morphological

characterization (Figure 3). All DNA bulks were able to produce amplified fragments with all selected 31 SSR primers, of which 15 primers with uniform bands without any heterogenic banding patterns. (Figure 3 and Supplemental Figure 1).

DNA bulks (B3, B4 and B6) displaying heterogenic banding patterns or unusual stutter bands for some markers (e.g. RM515, Supplemental Figure 2) were expanded and PCR products resolved again on 8% PAGE (Figure 4; Supplemental Figure 3 and 4) along with other non-expanded bulks amplified with the same primer. DNA bulks displaying a single banding pattern were not expanded as all DNA samples contained in those bulks were assumed to be homozygous for that marker.

Allelic diversity

Three to six alleles per locus were amplified with the mean value of four by 31 SSR primers across the 32 Dahanala accessions resulting in a total of 124 alleles. Except for monomorphic primer RM255, which was discarded from further analysis, the other 30 markers produced a range of alleles among the bulks and individual accessions. RM202 primer had the highest polymorphism, yielding six alleles per locus. The lowest major allele frequency was observed for marker RM515 (0.303). Genetic diversity ranged between 0.169 (RM216; RM236) and 0.744 (RM515) with a mean value of 0.475. The PIC varied between 0.161 (RM216; RM236) and 0.697 (RM412) (Table 4).

Table 2. Microsatellite primer details (Gramene Microsat). Ch. No., Chromosome number; AT, optimized annealing temperature (°C). For the location of RM277 and RM279 on the chromosomes, see Supplemental Figure 5.

Primer	Forward	Reverse	Product size (bp)	Ch. No.	AT (°C)
RM20B	atcttgcctcgcaggtcat	gaaacagaggcacatttcattg	114–144	11	57
RM25	ggaaagaatgatctttcatgg	ctaccatcaaaaccaatgttc	120–124	8	57
RM84	taagggtccatccacaagatg	ttgcaaatgcagctagagtac	118–124	1	57
RM201	ctcgtttattacctacagtacc	ctacctctttctagaccgata	136–150	9	57
RM202	cagattggagatgaagtccctcc	ccagcaagcatgtcaatgta	166–186	11	59
RM207	ccattcgtgagaagatctga	cacctcatcctcgtaacgcc	110–132	2	57
RM208	tctgcaagccttgctgatg	taagtcgatcattgtgtggacc	164–176	2	57
RM213	atctgtttgcaggggacaag	aggtctagacgatgtcgtga	126–150	2	59
RM215	caaatggagcagcaagagc	tgagcacctccttctctgtag	146–160	9	59
RM216	gcatggccgatggtaaag	tgtataaaaccacacggcca	132–154	10	61
RM217	atcgcaagaatgcctcgt	gggtgtgaacaagacac	114–144	6	57
RM219	cgtcggatgatgtaaagcct	catatcggcattcgcctg	184–204	9	55
RM220	ggaaggtaactgtttccaac	gaaatgcttcccacatgtct	100–130	1	61
RM224	atcgatcgatcttcacgagg	tgctataaaaggcattcgagg	124–142	11	61
RM228	ctggccattagtccttg	gcttgccgctctgcttac	100–122	10	61
RM236	gcgctggtgaaaaatgag	ggcatcctctttgattcctc	190–196	2	57
RM237	caaatcccactgctgtcc	tgggaagagagcactacagc	124–138	1	57
RM241	gagccaaataagatcgctga	tgcaagcagcagatttagtg	124–142	4	57
RM255	tgttgctgttgagatgtg	cgaaccgctcagttcaac	132–154	4	57
RM259	tggagtttgagaggagg	cttggtgcatggtgccatgt	148–176	1	59
RM270	ggccgttggttctaaaatc	tgcgagtatcatcgcgag	210–224	12	57
RM277	cgtgcaaatcatcacctgac	caaggcttgaagggaag	118–124	12	55
RM279	gcgggagagggatctcct	ggctaggagttaacctgcg	148–174	2	55
RM412	cacttgagaaagttagtgacg	cccaaacacaccaataac	176–200	6	57
RM418	tcgcgtatcgtcatgcatag	gagcacatatgccacgtacg	245–290	7	59
RM440	catgcaacaacgtcaccttc	atgggttgtaggcaccaaag	161–217	5	59
RM480	gtcaagcattctgcagttg	gcgcttctgcttattggaag	199–221	5	61
RM515	taggacgaccaaagggtgag	tggcctgctctctctctc	211–219	8	57
RM518	ctcttactactaccatgg	atccatctggagcaagcaac	158–180	4	55
RM536	tcttctctctgtttggtc	acacaccaacacgaccacac	220–230	11	61
RM539	gagcgtcctgtttaaaccg	agtagggtatcacgcatccg	249–289	6	61
RM560	gcaggaggaacagaatcagc	agcccgatgacggtgatag	224–240	7	59
RM571	ggaggtgaaagcgaatcatg	cctgctgctcttcatcagc	182–192	3	61

Table 3. Variation of seed morphology of 32 Dahanala accessions. Grain shape: 2, semi-round shape; 3, half-spindle shape; 4, spindle shape. Lemma and palea colour: 0, straw; 3, brown furrows on straw; 4, brown; 9, black; 11, brownish-black; 18, brownish-black furrows on straw background. Seed coat color: 5, red. Sterile lemma color: 1, straw. Pubescence of lemma: 3, hair on upper portion, 4, short hairs. For the complete list of modified descriptors, see [Supplemental Data 1](#).

Accession No.	Grain shape	Lemma and palea colour	Seed coat colour	Sterile lemma colour	Pubescence of lemma	Grain width [mm]	Grain length [mm]
002049	4	0	5	1	3	3.218	9.314
002050	3	0	5	1	4	3.264	8.366
002053	4	3	5	1	4	3.186	8.336
003131	4	11	5	1	3	3.204	8.618
003149	3	11	5	1	3	3.084	8.250
003386	4	3	5	1	4	3.230	8.878
003540	4	3	5	1	3	3.480	8.466
003917	2	0	5	1	4	2.858	7.126
003924	3	9	5	1	4	3.386	7.912
003971	3	18	5	1	3	3.508	8.388
004030	4	3	5	1	4	3.304	8.584
006165	3	9	5	1	3	3.010	7.790
004968	3	11	5	1	3	2.866	7.158
006376	4	9	5	1	3	3.106	8.156
006377	3	0	5	1	3	3.346	6.946
006378	4	11	5	1	4	3.266	7.848
006739	3	11	5	1	3	3.204	7.880
010160	3	9	5	1	3	3.126	8.148
014122	4	4	5	1	4	3.366	9.620
015533	4	4	5	1	4	3.386	8.446
006357	4	3	5	1	4	2.880	8.772
006358	4	3	5	1	3	3.074	8.688
003304	3	11	5	1	3	3.454	8.654
003327	3	9	5	1	4	3.166	8.188
004507	3	11	5	1	4	3.094	8.364
592	3	3	5	1	3	3.252	8.580
626	3	11	5	1	4	3.256	8.848
627	3	11	5	1	4	3.436	8.340
629	3	11	5	1	3	3.198	8.082
1214	3	4	5	1	4	3.148	8.602
1246	3	4	5	1	3	3.204	8.126

Cluster analysis

A phylogenetic tree was constructed from Nei’s genetic distances and showed significant genetic diversity within the population by grouping all accessions into six major clusters ([Figure 5](#)). Some variation of the clustering pattern was observed compared to previously classified eight bulks based on morphology characterization. Bulks 1, 2 and 8 from morphological characterization, along with control Bg 360 clustered together, also bulks 6 and 7 formed a single cluster. On the other hand, bulk 4 is separated into two sub-clusters based on molecular characterization. Furthermore, phylogenetic results revealed accessions 003924/003327 and accessions 0010160/006165 as two sets of putative duplicates among the 32 Dahanala accessions, suggesting the possibility of having duplicated samples among conserved accessions.

Trichome analysis

The 32 Dahanala accessions were characterized for their trichome morphology and density as a proxy for potential thrips resistance. A significant variation in trichome density within the population was identified ($P < 0.05$) ([Table 5](#)). The highest and the lowest density values were recorded from accession 003386 (30.83 trichomes/mm²) and accession 006357 (6.70 trichomes/mm²), respectively. The Suduru Samba variety, which is highly susceptible to thrips, had no trichomes ([Figure 6](#)) while moderately thrips susceptible Bg 360 showed medium trichome density (8 trichomes/mm²) ([Table 5](#)).

Besides the density variation, differences in nature and the placements of the trichomes on the adaxial surfaces of the rice leaves were observed under the light microscope ([Figures 7 and 8](#)).



Figure 1. Seed morphological characters of Dahanala red rice accessions. Left: hulled seeds; Right: dehulled seeds. Acc. No.006739, reported to be the oldest line of Dahanala variety conserved at IRRI, is highlighted as a reference accession for morphological comparison with seeds of other accessions.

Evaluation of the presence of RM277 and RM279 in selected Dahanala accessions

Based on previous studies of [Gimhani \(2010\)](#), RM277 and RM279 primers, selected for having a putative relationship with thrips resistance and trichome density, were used for PCR amplification of selected accessions. Ten accessions were selected to represent all 32 accessions of Dahanala based on the calculated trichome density values and significantly different morphology of the trichomes. These included two accessions with long trichomes (Acc. No. 003924; Acc. No. 003304), one accession with short trichomes (Acc. No. 002049), two accessions with the highest and lowest trichome density (Acc. No. 003386 and 006357, respectively), one

accession with comparatively bigger gaps in between two trichomes (Acc. No. 592), and one accession which deviated in the cluster analysis (Acc. No. 003971). Moderately thrips-susceptible Bg 360 and highly thrips-susceptible Suduru Samba (two accessions, see [Table 1](#)) were used as reference samples.

No polymorphism was observed in amplified PCR products of RM277 between the Bg 360 sample and the selected Dahanala accessions with lengthy trichomes (Acc. No. 003924; Acc. No. 003304), the accession with the lowest trichome density (Acc. No. 006357), the accession with the highest trichome density (Acc. No. 003386), the accession with short trichomes (Acc. No. 002049), the accession with comparatively higher gaps between two trichomes (Acc. No. 592), and the

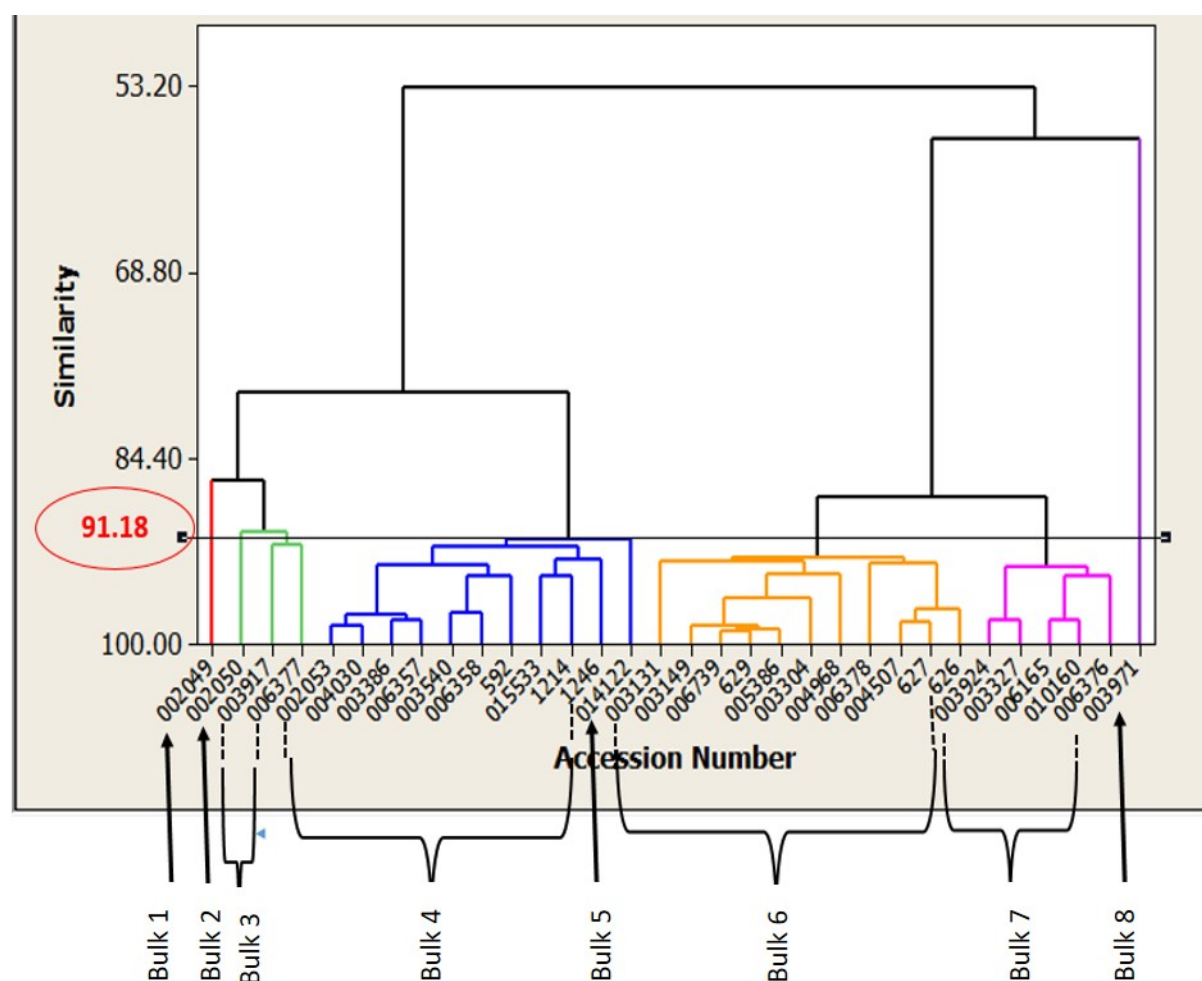


Figure 2. Dendrogram of 32 Dahanala rice accessions based on seed morphological characterization. Accession numbers are as in Table 1. The grouping at 91.18% similarity level used to generate DNA bulks for molecular characterization is outlined on the bottom.

accession that revealed to be a deviated accession in cluster analysis (Acc. No. 003971) (Figure 8). However, the highly susceptible Suduru Samba variety (zero trichomes) was polymorphic in this marker compared to other samples.

Discussion

In this study, we analyzed the genetic diversity among 32 accessions of the traditional rice variety Dahanala using seed morphology and 31 SSR markers, followed by a molecular and phenotypic evaluation of trichomes characters potentially related to thrips resistance. Since the morphological characterization was based on only seven seed traits, comparatively, a molecular diversity assessment using 31 loci present in the genome can provide more accurate results with higher reliability than morphological markers (Semagn, 2002).

The majority of the markers showed allelic diversity with a relatively high PIC value around 0.5, suggesting they can be used in future genetic diversity assessments of rice germplasm (Table 4). Interestingly, a previous study using the same SSR markers (Manatunga *et al*, 2019) also had PIC values ranging from 0.00 (RM518

and RM237) to 0.72 (RM515) denoting high genetic diversity but for different markers.

Similar to other Sri Lankan traditional varieties such as Pachchaperumal, Murungakayan, Pokkali, Kuruluthuda and Kaluheenati, Dahanala germplasm showed a significant genetic variation among the available 32 accessions, ranging from 0.0 to 0.94 compared to varieties Murungakayan (0.00–0.76) and Pokkali (0.33–0.77) (Siriwardhana *et al*, 2016; Warusawithana *et al*, 2017; Manatunga *et al*, 2019).

Our study found two pairs of putative duplicate accessions. Studies of Murungakayan, Pokkali and Kaluheenati varieties have found four sets of duplicates with the highest genetic similarities in their respective germplasms (Siriwardhana *et al*, 2016; Thotago-dawatta *et al*, 2017; Manatunga *et al*, 2019). Concurrently, Karunadasa and Samarasinghe (2017) also identified one set of genetically similar duplicates in Kuruluthuda.

Since the morphological characterization was based on only seven traits, comparatively, molecular diversity assessment can provide more contrasting results with higher reliability because DNA markers cover a

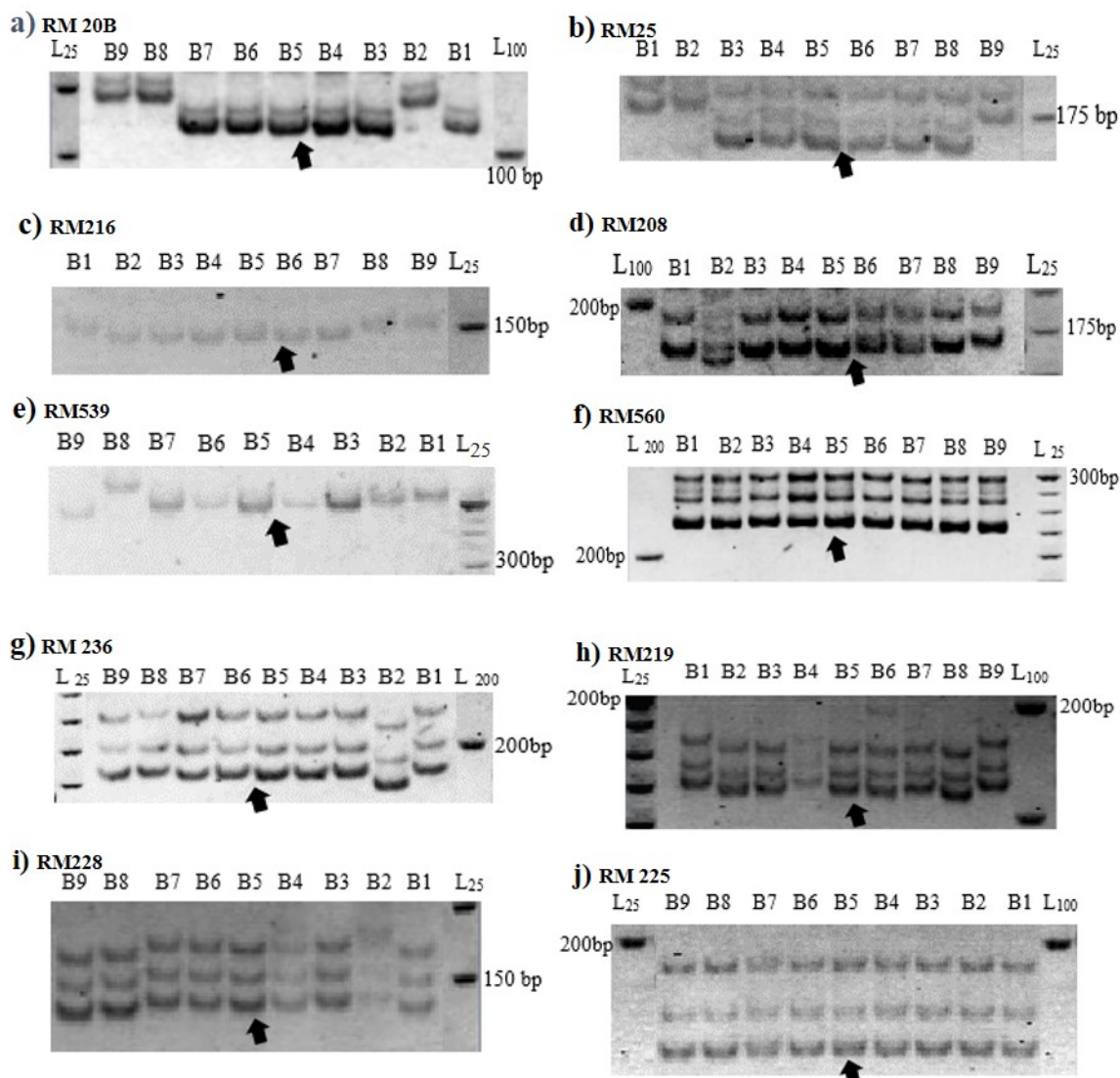


Figure 3. Polyacrylamide gel profiles (8%) showing the amplification of DNA bulks using 10 SSR primers. The arrowheads indicate corresponding scored alleles. B1–B8, DNA bulks; B9, Bg360; L25, L100, L200, size markers; bp, basepairs. For additional SSR primers see [Supplemental Figure 1](#).

major portion of the genome than morphological markers ([Semagn, 2002](#)).

According to the constructed phylogenetic tree, unexpectedly, the reference sample Bg 360 clustered with three Dahanala accessions (003971, 002050 and 002049) indicating a closer relationship of these samples respective to other accessions. Separate clustering of these three accessions was also observed under morphological characterization. Similar results were reported from the assessment of 23 Murungakayan accessions, where the Bg 360 reference sample clustered separately with Acc. No. 003495, exhibiting a genetic deviation from other accessions ([Manatunga et al, 2019](#)). [Matsui and Kagata \(2003\)](#) suggest that higher genetic variation could exist among individual accessions of the same cultivar because of cross-pollination along with temperature stress and some mutagenic characteristics of floral organs. Additionally, the accidental mixing of seed samples during the pro-

cess of sample collection and storage in genebank may cause some deviation.

In the present study, 16 out of 32 accessions were selected as a representative set of Dahanala, which group together in Cluster 1 with the highest similarity and carry the highest distance from the reference sample Bg 360. These include PGRC accessions 003924, 003327, 006376, 010160, 006165, 006378, 004968, 003304, 006739, 005386, 004507, 003149, 003131 along with three RRDI accessions 627, 626, 629. Most importantly, those 16 accessions clustered together with the Acc. No. 006739, which is considered the oldest line of Dahanala found in Sri Lanka ([Figure 6](#)). Similarly, in previous studies, 11 out of 20 accessions and 8 out of 23 accessions were selected as representative sets of Pachchaperumal and Murungakayan, respectively ([Warusawithana et al, 2017](#); [Manatunga et al, 2019](#)).

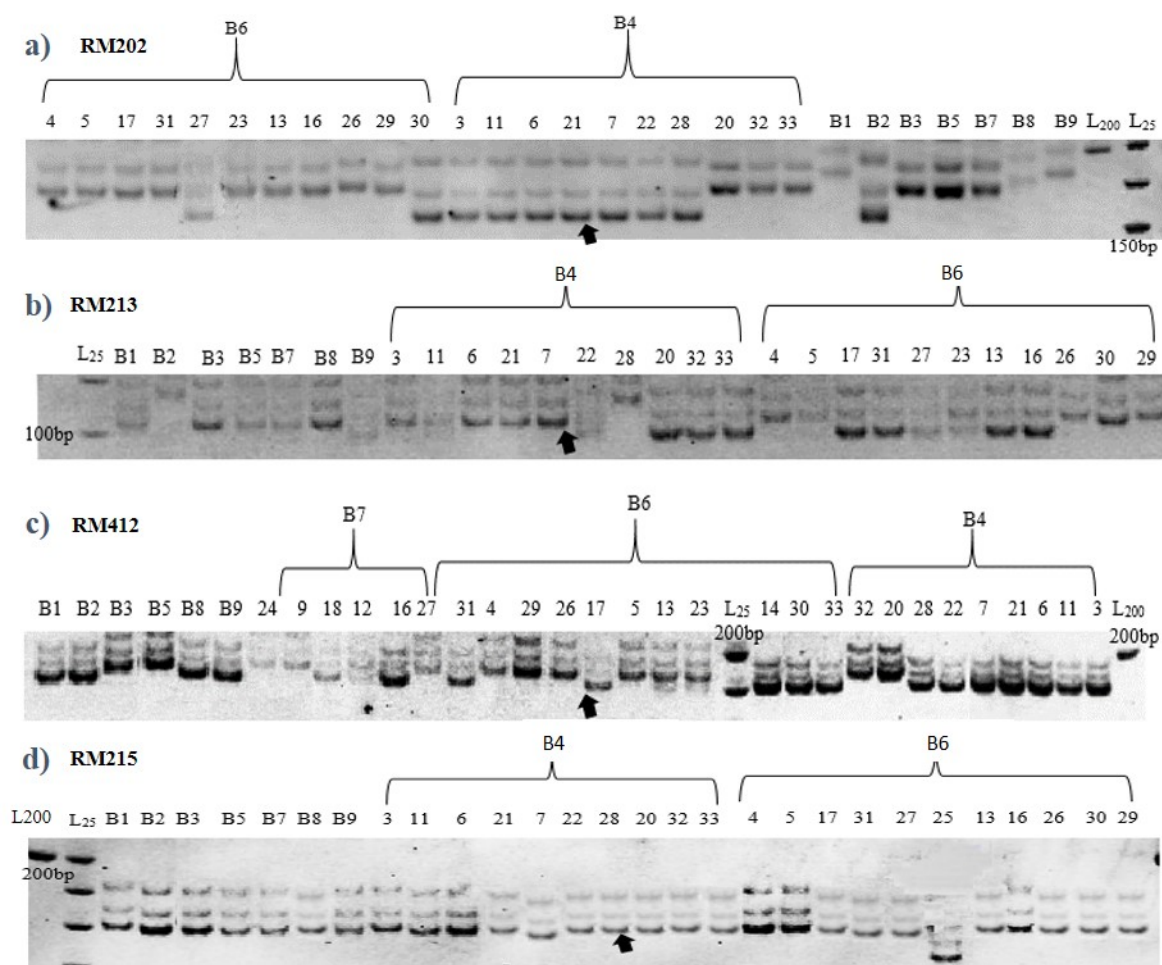


Figure 4. Amplification of individual accessions of expanded DNA bulks using four SSR primers. PCR products were separated on 8% PAGE along with other bulks. B, bulks; L25, L100, L200, size markers; bp, basepairs. Numbers indicate individual accessions within bulks (as in Table 1). Additional markers are included in Supplemental Figures 3 and 4.

As the morphological characterization is based on only seven seed morphology related traits, comparatively, the molecular diversity assessment, which carries an assessment of 31 loci present in the genome, provided more accurate results with higher reliability. Present results can be useful in selecting suitable accessions of Dahanala as donor parents in future rice breeding programmes. However, with respect to conserving germplasm of the Dahanala traditional rice variety, further studies should be conducted to verify the identity of this representative sample of Dahanala via amplification of a higher number of loci or sequencing the genome.

Trichome analysis

Since previous studies have revealed a relationship of thrips resistance with the presence of non-glandular trichomes on the adaxial surface of the leaves (Nugaliyadde and Heinrichs, 1984), and thrips reported to be fed on the mesophyll cells of the young leaves through the adaxial surface (Gimhani, 2010), our study focused on inspection of the trichome density on the adaxial surface of Dahanala, which is known as a thrips-resistant variety.

Even though all examined accessions were conserved in genebanks under the same variety name, we detected a significant variation among trichome densities as well as in the trichomes' nature and locality among accessions (Figures 6 and 7).

In a first attempt at identifying QTLs for thrips resistance in rice, Gimhani (2010) identified both thrips resistance and trichome density as quantitative traits based on bulk segregating analysis followed by a screening of an F_2 population between thrips-resistant Dahanala and susceptible Suduru Samba. Gimhani (2010) found a significant negative correlation between thrips damage and the trichome density values ($r = -0.378$, $P < 0.05$), revealing the damage score is low when the trichome density is high. They also showed that 14.3% of phenotypic variation (thrips resistance) in F_2 segregants could be explained by a factor of presence of trichomes, suggesting that the presence of trichomes could play a significant role in defending the plants against thrips, in agreement with the findings of Nugaliyadde and Heinrichs (1984), who reported that morphological features of the foliage and the presence of allomones in resistant plants contribute to their defence against thrips. In addition, Ananthakrishnan (1979)

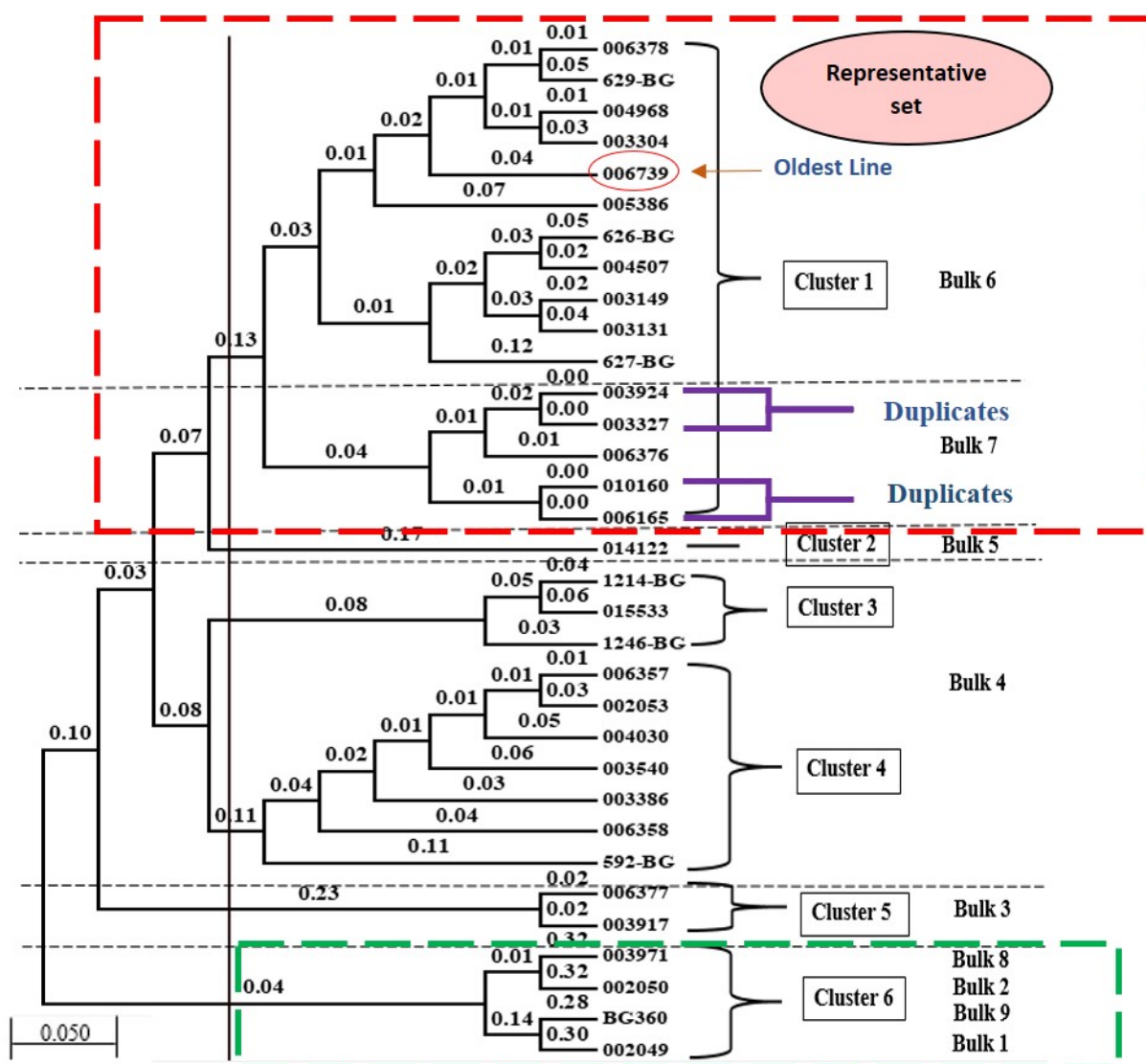


Figure 5. Dendrogram of Dahanala accessions based on analysis of simple sequence repeat (SSR) markers. Branch lengths are indicated by decimal values on the branches. Bulks based on morphological clustering are indicated. Duplicates and reference accessions are indicated. The representative set of Dahanala accessions is indicated by a red-dotted rectangle. The deviated bulks are indicated by the green-dotted rectangle. BG, Batalagoda accessions.

found that leaf age and thickness, and the nature and distribution of trichomes and silica cells in the substrate are some important biophysical factors that influence thrips oviposition. On the other hand, Wickramasinghe *et al* (2007), showed that the removal of trichomes on the adaxial surface of the leaf of Dahanala seedlings did not result in a highly susceptible reaction. This indicates that besides trichome density there might be several other factors influencing thrips resistance. Based on the analysis of this study, the nature along with the position of trichomes on the leaf can also be one of those factors.

Although an association of trichome density with RM277 and RM279 markers was identified previously (Gimhani, 2010), the results of the current project were not able to provide clear evidence of this relationship, as the Dahanala accessions used in this study were not polymorphic for RM277 and the allelic patterns for RM279 of both accessions with the highest (003386; 30.83 trichomes/mm²) and lowest (006357;

6.70 trichomes/mm²) trichome density were identical. Additionally, as our current study only analyzed selected representative samples of Dahanala using RM279 and RM277 putative markers, the results are insufficient to predict a clear association. Hence additional studies will be required to further test this hypothesis.

Although marker RM277 did not show polymorphism between selected Dahanala accessions and the Bg 360 variety, the highly thrips-susceptible Suduru Samba varieties with zero trichomes were polymorphic for this marker. Therefore, RM277 can be suitable for use in future analysis to detect Suduru Samba against the Dahanala variety. Additionally, since significant allelic variation was discovered within all selected Dahanala accessions as well as among Dahanala, Suduru Samba, and Bg 360 varieties with RM279 marker, this evidence can be used to select among these groups in future studies.

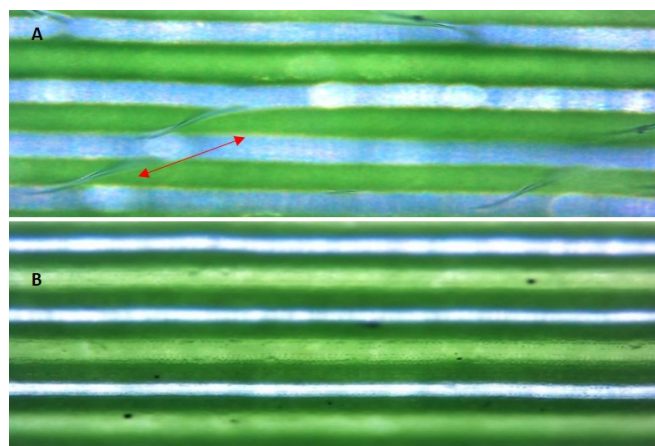


Figure 6. Microscopic images of trichomes on adaxial surfaces of rice leaves of reference samples. A, Bg 360, moderately susceptible to thrips; B, Suduru Samba, highly susceptible to thrips (without any trichomes on the adaxial surface; zero trichomes). The red arrow indicates the presence of trichomes.

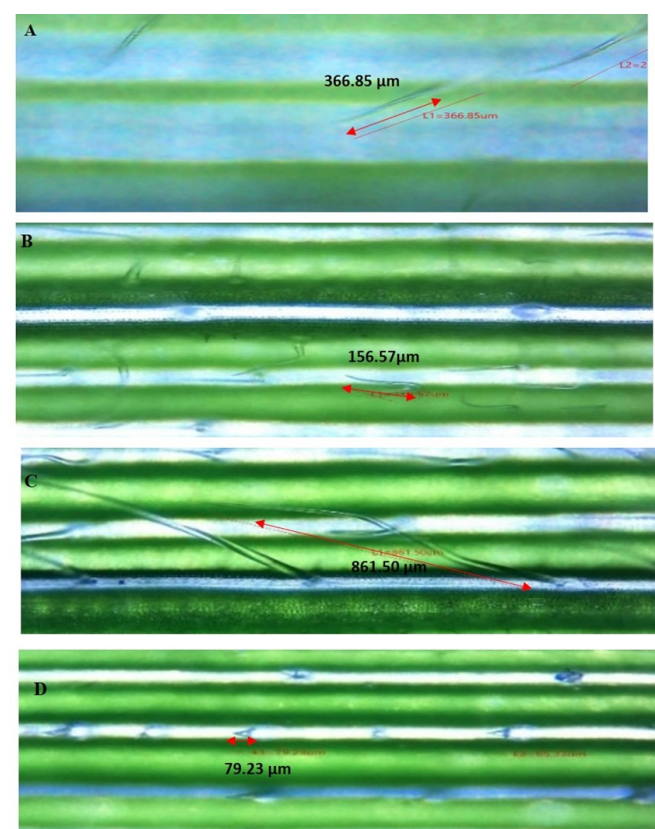


Figure 7. Microscopic images of trichomes on adaxial surfaces of Dahanala rice leaves denoting variance among accessions. A, Acc.No. 006357, the accession with the lowest trichome density (7.0 trichomes/mm²); B, Acc. No. 003386, the accession with the highest trichome density (31.0 trichomes/mm²); C, Acc. No. 003304, the accession with the longest trichomes (~860µm); D, Acc.No. 002049, the accession with the shortest trichomes (~79µm). The red arrows indicate the presence of trichomes.

Table 4. Allelic diversity of 31 SSR markers on 32 traditional red rice accessions. PIC, polymorphic information content.

Marker	Major Allele Frequency	Allele No.	Gene Diversity	PIC
RM20B	0.879	3	0.219	0.204
RM25	0.909	4	0.171	0.166
RM84	0.667	4	0.517	0.481
RM201	0.515	4	0.608	0.537
RM202	0.394	6	0.722	0.677
RM207	0.758	5	0.408	0.386
RM208	0.530	4	0.538	0.435
RM213	0.515	4	0.612	0.544
RM215	0.485	5	0.623	0.553
RM216	0.909	3	0.169	0.161
RM217	0.636	4	0.541	0.497
RM219	0.606	3	0.500	0.401
RM220	0.515	5	0.630	0.572
RM224	0.606	5	0.579	0.538
RM228	0.576	3	0.533	0.442
RM236	0.909	3	0.169	0.161
RM237	0.606	3	0.533	0.458
RM241	0.879	4	0.222	0.214
RM255	1.000	1	0.000	0.000
RM259	0.515	4	0.553	0.456
RM270	0.515	3	0.551	0.452
RM412	0.333	5	0.740	0.698
RM418	0.697	5	0.490	0.467
RM440	0.515	5	0.669	0.631
RM480	0.879	3	0.220	0.209
RM515	0.303	4	0.744	0.696
RM518	0.364	5	0.736	0.692
RM536	0.576	5	0.604	0.560
RM539	0.879	4	0.222	0.214
RM560	0.545	3	0.544	0.448
RM571	0.788	5	0.365	0.348
Mean	0.639	4	0.475	0.429

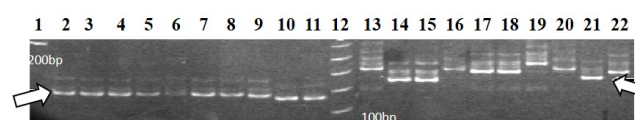


Figure 8. Allelic variation for RM277 and RM279 primers in selected Dahanala red rice accessions. Lanes 2–11, PCR product amplified with RM277; Lanes 1 3–22, PCR product amplified with RM279; 1, 200bp ladder; 2, Acc. No. 003924; 3, Acc. No. 006357; 4, Acc. No. 003386; 5, Acc. No. 002049; 6, Bg360 (moderately susceptible); 7, Acc. No. 592; 8, Acc. No. 003304; 9, Acc. No.003971; 10, Suduru Samba Batalegoda variety; 11, Suduru Samba PGRC variety Acc. No 003333; 12, 25bp ladder; 13, Acc. No. 003304; 14, Suduru Samba PGRC variety Acc. No. 003333; 15, Suduru Samba Batalegoda variety; 16, Acc. No. 003924; 17, Acc. No. 006357; 18, Acc. No. 003386; 19, Acc. No. 002049; 20, Bg 360 (Moderately susceptible); 21, Acc. No. 003971; 22, Acc. No. 592.

Table 5. Trichome density observed for 32 Dahanala traditional red rice accession. Accessions were grouped as follows: A, 31–35 trichomes/mm²; B, 26–30 trichomes/mm²; C, 21–25 trichomes/mm²; D, 16–20 trichomes/mm²; E, 11–15 trichomes/mm²; F, 5–10 trichomes/mm²; G, 0–5 trichomes/mm².

Accession No.	Cultivar Name	Density (trichomes/mm ²)	Group
3386	Dahanala	31	A
15533	Dahanala	26	B
626	Dahanala	26	B
2049	Dahanala	25	C
3131	Dahanala 2014	23	C
3149	Dahanala 37 YM	23	C
3924	Dahanala	23	C
10160	Dahanala	23	C
14122	Dahanala	23	C
5386	Dahanala	22	C
3917	Dahanala	21	C
6377	Dahanala	21	C
3304	Danahala	21	C
3327	Danahala	21	C
6376	Dahanala	20	D
3540	Dahanala	19	D
6378	Dahanala	19	D
6739	Dahanala	19	D
2053	Dahanala	18	D
3971	Dahanala	18	D
4968	Dahanala	18	D
629	Dahanala	18	D
4507	Dhana Hala	17	D
627	Dahanala	17	D
1214	Dahanala	16	D
2050	Dahanala	15	E
4030	Dahanala	15	E
592	Wanni Dahanala	14	E
1246	Dahanala	14	E
6165	Dahanala	13	E
6358	Wanni Dahanala	11	E
6357	Wanni Dahanala	7	F
	Bg 360	8	F
	Suduru Samba	0	G

One reason for not detecting a strong association between trichome density and RM279 or RM277 markers in this research might be some limitations associated with the study, such as the low number of accessions studied. Therefore, before selecting a donor parent for thrips resistance among Dahanala accessions, further investigations, including disease

assays, are required to corroborate the relationship between trichome density, RM277 and RM279 markers and a possible thrips resistance.

Acknowledgements

The authors would like to express their sincere gratitude towards Dr W. L. G. Samarasinghe and Dr S. K. Wasala including all the staff members of the Biotechnology division, PGRC, Gannoruwa; Peradeniya, RRDI; Batalagoda, Sri Lanka, and the Department of Biotechnology, Wayamba University of Sri Lanka.

Supplemental data

Supplemental Data 1: Description of seed morphological characters.

Supplemental Figure 1: Additional SSR marker profiles amplified from DNA bulks (RM201, R M220, RM241, RM259, RM270).

Supplemental Figure 2: PCR profile or heterogenic marker RM515 of DNA bulks.

Supplemental Figure 3: PCR profiles of expanded DNA bulks for SSR markers (RM84, RM207, RM224, RM480, RM518, RM536).

Supplemental Figure 4: PCR profiles of expanded DNA bulks for SSR markers (RM217, RM237, RM418, RM440, RM515, RM571).

Supplemental Figure 5: Map position of markers RM277 and RM279 on rice chromosomes 12 and 2.

Author contributions

All authors contributed to the study's conception and design. V. Koodalugodaarachchi prepared the material, the experiment, and the data collection, and wrote the first draft. D. S. Kekulandara and D. R. Gimhani provided advice on the experiment conduction and data analysis, and commented on the first and second drafts. The final manuscript was read and approved by all authors.

Conflict of interest statement

The authors declare no conflict of interest.

References

- Ananthakrishnan, T. N. (1979). Biosystematics of Thysonoptera. *Annual Review of Entomology* 24, 159–183. doi: <https://doi.org/10.1146/annurev.en.24.010179.001111>
- Bioversity International, IRRI and WARDA (2007). Descriptors for wild and cultivated rice (*Oryza* spp.)(Rome, Italy: Bioversity International). doi: <https://hdl.handle.net/10568/72595>
- Gimhani, D. R. (2010). Identification of Microsatellite Marker Linked to Thrips (*Stenchaetothrips biformis*) Resistance in Rice Using Bulk Segregant Analysis. Ph.D. thesis, University of Peradeniya, Sri Lanka.
- (Gramene). Introduction to Rice. url: https://archive.gramene.org/species/oryza/rice_intro.html.

- (Gramene Microsat). All SSRs. url: <https://archive.gramene.org/markers/microsat/all-ssr.html>.
- Jeffreys, A. J., Brookfield, J. F., and Semeonoff, R. (1985). Positive identification of an immigration test-case using human DNA fingerprints. *Nature* 317, 818–819. doi: <https://doi.org/10.1038/317818a0>
- Karunadasa, R. M. S. P. and Samarasinghe, W. L. G. (2017). Genetic diversity analysis of traditional rice variety 'Kuruluthuda' using SSR markers. In Proceeding of the International Research Symposium, Uva Wellassa University, Sri Lanka.
- Korzun, V. (2002). Use of molecular markers in cereal breeding. *Cellular and molecular biology letters* 2, 811–820. url: https://www.researchgate.net/profile/Viktor-Korzun/publication/11082462_Use_of_molecular_markers_in_cereal_breeding/links/09e41507f31792683b000000/Use-of-molecular-markers-in-cereal-breeding.pdf.
- Kudagamage, C. (1977). Varietal resistance to the rice thrips, *Baliothrips biformis*. *International Rice Research Newsletter* 2, 11–11. url: <http://books.irri.org/IRRN2no5.content.pdf>.
- Liu, K. and Muse, S. V. (2005). PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics* 21(9), 2128–2129. doi: <https://doi.org/10.1093/bioinformatics/bti282>
- Manatunga, M. M. S. L., Wasala, S. K., Sumanasinghe, V. A., and Ubeysekara, N. M. (2019). Genetic diversity analysis of 'Murungakayan' Rice accessions based on seed morphology and molecular characterization. *Tropical Agricultural Research* 30(2), 65–73. doi: <http://dx.doi.org/10.4038/tar.v30i2.8310>
- Matsui, T. and Kagata, H. (2003). Characteristics of floral organs related to reliable self-pollination in rice (*Oryza sativa* L.). *Annals of Botany* 91(4), 473–477. doi: <https://doi.org/10.1093/aob/mcg045>
- McCouch, S. R., Temnykh, S., Lukashova, A., Coburn, J., Declerck, G., Cartinhou, S., Harrington, S., Thomson, M., Septiningsih, E., Semon, M., Moncada, P., and Li, J. (2001). Microsatellite markers in rice: abundance, diversity, and applications. In *Rice genetics IV. Proceedings of the Fourth International Rice Genetics Symposium, 22-27 October 2000, Los Baños, Philippines.*, ed. Khush, G. S., Brar, D. S., Hardy, B., et al., (Enfield, NH, USA.: Science Publishers, Inc.), 117–135.
- Minitab (1991). Minitab reference Manual (Release 7.1) (State College, PA 16801, USA: Minitab Inc).
- Murray, M. G. and Thompson, W. F. (1980). Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research* 8(19), 4321–4326. doi: <https://doi.org/10.1093/nar/8.19.4321>
- Nei, M., Tajima, F., and Tateno, Y. (1983). Accuracy of estimated phylogenetic trees from molecular data. *Journal of Molecular Evolution* 19, 153–170. doi: <https://doi.org/10.1007/BF02300753>
- Nugaliyadde, L. and Heinrichs, E. A. (1984). Resistance of *Oryza* spp. to thrips, *Stenchaetothrips biformis* (Baginall) (Thysanoptera: Thripidae). *Crop Protection* 3, 305–313. doi: [http://dx.doi.org/10.1016/0261-2194\(84\)90036-X](http://dx.doi.org/10.1016/0261-2194(84)90036-X)
- Panda, N. and Khush, G. B. (1995). Host Plant Resistance of Insects: Mechanisms of Resistance (United Kingdom: Biddles Ltd).
- Semagn, K. (2002). Genetic relationships among ten endod types as revealed by a combination of morphological, RAPD and AFLP markers. *Hereditas* 137(2), 149–156. doi: <https://pubmed.ncbi.nlm.nih.gov/12627842/>
- Shahbandeh, M. (2022). Rice - statistics & facts. Statista.
- Siriwardhana, S. M., Samarasinghe, W. L. G., and Gimhani, D. R. (2016). Molecular characterization of a traditional rice variety 'Kaluheenati' (*Oryza sativa* L.) using Simple Sequence Repeats markers. In Proceedings of 15th Agricultural Research Symposium, 135–139.
- Suriyagoda, L. D. B., Thilakarathne, R. M. M. S., Nissanka, S. P., and Samita, S. (2011). Morphological variation in selected rice (*Oryza sativa* L.) germplasm of Sri Lanka. *Journal of the National Science Foundation of Sri Lanka* 39, 129–137. doi: <http://doi.org/10.4038/jnsfsr.v39i2.3173>
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30(12), 2725–2729. doi: <https://doi.org/10.1093/molbev/mst197>
- Thotagodawatta, T. M. N. B. K., Samarasinghe, W. L. G., Alwis, L. M. H. R., and Ubeyasekara, N. M. (2017). Genetic diversity analysis of traditional rice variety 'Pokkali' using Simple Sequence Repeat markers (SSR). In Proceedings of the International Research Symposium, Uva Wellassa University, Sri Lanka, 190p.
- Warusawithana, T. M., Samarasinghe, W. L. G., Das-sanayake, P. N., Ubeysekara, N. M., and Jayarathna, K. G. C. N. (2017). Genetic Diversity Analysis of Traditional Rice Variety 'Pachchaperumal' Using SSR Markers. *International Journal of Scientific and Technology Research* 6(8), 229–237. url: <https://www.ijstr.org/final-print/aug2017/Genetic-Diversity-Analysis-Of-Traditional-Rice-Variety-pachchaperumal-Using-Ssr-Markers.pdf>.
- Wickramasinghe, H. A. M. and Noda, T. (2008). Physicochemical properties of starches from Sri Lankan rice varieties. *Food Science and Technology Research* 14, 49–54. doi: <http://dx.doi.org/10.3136/fstr.14.49>
- Wickramasinghe, K. S., Nugaliyadde, P. K., Samara-jeewa, P. K., Rajapakse, R. M. T., and Ahangama, D. (2007). Effect of leaf trichomes to thrips resistance in rice. *Annals of Sri Lanka Department of Agri-culture* 9, 189–198. url: https://issuu.com/afacipdf/docs/effect_of_leaf_trichomes_to_thrips.



Stylosanthes scabra: genetic variability of forage quality traits

Fabiana Karla de Araújo Américo^a, Marcelo Ayres Carvalho^b, Juaci Vitória Malaquias^b, Allan Kardec Braga Ramos^b, Gustavo José Braga^b, Claudio Takao Karia^b and Carlos Eduardo Lazarini da Fonseca^{*,b}

^a Universidade Estadual de Feira de Santana-UEFS, Bahia, Brazil

^b Embrapa Cerrados, Planaltina, DF, Brazil

Abstract: *Stylosanthes scabra* Vogel is a tropical legume grown in dry tropical and subtropical environments. The objective of this research was to evaluate the genetic diversity of forage quality traits for 80 accessions of *S. scabra*. Seven plants from each accession were planted in a single-line plot with no replicates at Embrapa Cerrados, Brazil. All plants were harvested 90 days after planting. Crude protein (CP), *in vitro* dry matter digestibility (IVDMD), neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin (LIG), hemicellulose (HEMIC) and cellulose (CELLU) were estimated. Data were submitted to principal component analysis (PCA) and a cluster analysis was performed to identify groups of similarity. Simpson and Shannon–Weaver diversity indices estimated the genetic diversity. The average values of CP, IVDMD, NDF, ADF, LIG, HEMIC and CELLU were 220g/kg, 560g/kg, 516.8g/kg, 368g/kg, 69.4g/kg, 148.8g/kg and 298.6g/kg, respectively. There was a significant difference among collection sites for IVDMD, ADF and CELLU. The first two principal components accounted for 73% of the total variation. The 80 accessions resulted in four clusters, among which significant differences were observed for CP, IVDMD and ADF. Group IV, with 24 accessions, had the highest CP and IVDMD concentrations and the lowest ADF concentration, being the highest-quality forage group. Diversity indices were 0.78 and 0.81 for Simpson's and Shannon–Weaver's, respectively. In conclusion, there is genetic diversity for forage quality traits among *S. scabra*.

Keywords: Tropical legume, Genetic resources, Nutritional value, Diversity index, Germplasm characterization

Citation: de Araújo Américo, F. K., Carvalho, M. A., Vitória Malaquias, J., Braga Ramos, A. K., Braga, G. J., Karia, C. T., Lazarini da Fonseca, C. E. (2022). *Stylosanthes scabra*: genetic variability of forage quality traits. *Genetic Resources* 3 (5), 24–35. doi: [10.46265/genresj.UPPQ3994](https://doi.org/10.46265/genresj.UPPQ3994).

© Copyright 2022 the Authors.

This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

Brazil is a major beef producer and exporter, with an estimated herd of 171.8 million heads and about 158 million hectares of pastures (18.7% of Brazilian territory), of which 70% are cultivated and 30% are natural (IBGE, 2017). Most of the livestock farming in Brazil is still in extensive form, with the use of forage crops for livestock direct feeding into pasture (Hoffmann *et al*, 2014). Most production systems are based on the monoculture of tropical grasses of African origin. The use of high-quality forages improves the profitability

of production systems; an option to obtaining pastures with a high nutritional value is the introduction of legumes associated with grasses.

Legumes play an important role in animal production due to their high protein concentration compared to grasses, in addition to soil biological nitrogen fixation (Neres *et al*, 2012). Several studies showed the associated use of legumes and grasses enables a significant increase in the nutritional value of ruminants' diet (Carvalho and Pires, 2008).

When working with genetic resources it is very important to evaluate the genetic diversity of a set of germplasm, which will allow genetic improvement and the efficient use of a species' available germplasm.

*Corresponding author: Carlos Eduardo Lazarini da Fonseca (carlos.lazarini@embrapa.br)

Germplasm characterization include studies of eco-geographic and demographic adaptation, plant genetics, physiology and pathology, parameters of an organism's vital cycle and yield evaluation, among other studies. Breeding programmes should begin only after appropriate germplasm characterization (Cameron, 1983).

Characterization is the best way to understand the variability in a germplasm collection and, consequently, increase its use by plant breeders. It is also important in monitoring the genetic stability of germplasm storage processes. Characterization of germplasm can be based on molecular, biochemical, morphological, and agronomic features.

Despite the importance of forage legumes in various production systems, the adoption of tropical legume germplasm has been poor in several Latin American countries (Shelton *et al*, 2005; Boddey *et al*, 2020). However, according to Kretschmer (1988), there are about 18,000 species of forage legumes and at least 1,000–2,000 species with potential for cultivation.

The genus *Stylosanthes* includes nitrogen-fixing and drought-tolerant species economically important for perennial pasture, green manure and land recovery (Marques *et al*, 2018). Its importance as a forage crop has increased in recent years due to its potential to improve the nutritional quality of mixed pastures in tropical and subtropical regions (Cameron *et al*, 2004; Costa, 2006; Rocha, 2014; Epifanio *et al*, 2020; Carvalho *et al*, 2020). Due to its easy adaptation to acid and low-fertility soils, it has been used in pastures to minimize nitrogen deficiency in the soil, increase protein content in the diet, as well as improve pasture carrying capacity, longevity and productivity (Resende *et al*, 2008; Rocha, 2014).

Embrapa Cerrados has a germplasm bank (AGB) with thousands of accessions of the genus *Stylosanthes*, of which 80 are from *Stylosanthes scabra* Vogel species. Genetic resources conservation and characterization are of utmost importance to support breeding programmes for incorporating novel agronomic traits and developing new cultivars (Wetzel *et al*, 2012; Moreira *et al*, 2015).

Genetic resources characterization has been used to quantify genetic diversity, its magnitude, nature and distribution among and within populations (Boldt, 2011; Cruz *et al*, 2011). The evaluation of the nutritional quality diversity of *S. scabra* accessions can contribute to expanding the use of these genetic resources, and provides important information to high-quality forage breeding programmes (Pereira *et al*, 2011). Thus, the objective of this study was to access the genetic diversity of forage quality traits among 80 accessions of *S. scabra* from the Embrapa Cerrados active germplasm bank.

Materials and methods

Experimental setup

An experiment was carried out at the Embrapa Cerrados Research Centre, in Planaltina, Federal District, Brazil (15° 35' S, 47° 42' W; 993m a.s.l.), from December 2017

to April 2018, under irrigated conditions, to evaluate the genetic variability of accessions of *S. scabra*. The climate at the experimental site is tropical savannah according to the Köppen–Geiger classification (Peel *et al*, 2007). The experiment was planted in clay soil (Rhodic Haplustox Oxisol) with, on average: pH 5.1, organic matter concentration of 29g/kg, K concentration of 46mg/kg, Al concentration of 25mg/kg and P concentration of 2.2mg/kg (Mehlich-I) at 0–0.2m soil depth.

Seeds from all samples were treated by mechanical scraping with 100-grit sandpaper and sown in polyethylene trays with 60 cells of 230cm³ filled with a commercial substrate. Fifty-day old seedlings were planted in single-line plots spaced 0.5m within and 1.0m between plots. Each plot consisted of seven plants from a specific accession.

Plant material

Eighty accessions of *S. scabra* collected in 9 different States from 4 distinct geographical regions in Brazil were evaluated: 26 from the States of Goiás (GO), Mato Grosso (MT), and Mato Grosso do Sul (MS) in the Central-West Region; 10 from Pará (PA) and Tocantins (TO) in the North Region; 36 from Bahia (BA) and Maranhão (MA) in the Northeast Region; and 8 from Minas Gerais (MG) and Rio de Janeiro (RJ) in the Southeast Region. Detailed information on each accession is presented in Table 1.

Data collection and analysis

All plants were harvested 90 days after transplanting to the field at 10cm stubble height. After an individual morphological plant characterization, all seven plants from each accession were pooled together, dried for 72 hours in a forced-air oven at 55°C, ground through a 1mm-screen Wiley mill (A. H. Thomas Co., Boulder, CO), stored and sealed in plastic containers, and sent to the Analytical Plant Chemistry Laboratory at Embrapa Cerrados.

The samples were then analyzed to estimate the content of neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin (LIG), crude protein (CP) and *in vitro* dry matter digestibility (IVDMD) on dry matter (DM) basis. DM content of the samples was determined by drying approximately 2g of each sample in a forced-air oven at 105°C ± 2°C for at least 2 hours (AOAC, 1996). Sequential NDF, ADF and LIG analysis from 0.5g initial sample weight placed in ANKOM F57 filter bags (Van Soest *et al*, 1991; Komarek and Sirois, 1993; Komarek *et al*, 1994; Vogel *et al*, 1999; Mertens, 2002) was performed for each accession sample. For NDF and ADF analysis, samples were digested in an Automated Fiber Analyzer model ANKOM 2000 (ANKOM Tech. Corp., Fairport, NY, USA) using a neutral detergent solution pH6.9–7.1, without α -amylase, and 1M H₂SO₄ acid detergent solution, sequentially after drying and weighing procedures. LIG determination was performed by placing the ADF dry residue in a 12M H₂SO₄ solution and incubated in a Tecnal *in vitro* incubator system

Table 1. Passport data of *Stylosanthes scabra* Vogel accessions from the Embrapa Cerrados germplasm bank. Embrapa Cerrados, Planaltina, DF, 2020. CPAC Number, Germplasm bank accession number. Brazilian States: BA, Bahia; GO, Goiás; MA, Maranhão; MG, Minas Gerais; MT, Mato Grosso; MTS, Mato Grosso do Sul; PA, Pará; RJ, Rio de Janeiro; TO, Tocantins. Brazilian Geographic Region: CO, Central-West; N, North; NE, Northeast; SE, Southeast.

Access ID	CPAC Number	Brazilian City/State	Brazilian Geographic Region	Latitude	Longitude
1	512	Rondonópolis-MT	CO	16° 18' 00" S	54° 45' 00" W
2	961	Rio de Janeiro-RJ	SE	22° 54' 06" S	43° 10' 27" W
3	963	Camaçari-BA	NE	12° 40' 60" S	38° 19' 00" W
4	964	Nazaré-BA	NE	13° 00' 00" S	39° 01' 60" W
5	965	Feira de Santana-BA	NE	12° 22' 00" S	39° 06' 00" W
6	966	Cruz das Almas-BA	NE	12° 40' 00" S	39° 06' 00" W
7	967	Mucuri-BA	NE	18° 04' 60" S	39° 34' 00" W
8	968	Pé de Serra-BA	NE	11° 49' 57" S	39° 36' 06" W
9	969	Feira de Santana-BA	NE	12° 16' 00" S	39° 01' 60" W
10	970	Santo Amaro-BA	NE	12° 31' 88" S	38° 49' 60" W
11	971	Feira de Santana-BA	NE	12° 22' 00" S	39° 06' 00" W
12	972	Catu-BA	NE	12° 20' 60" S	38° 23' 60" W
13	973	Conceição do Almeida-BA	NE	12° 55' 00" S	39° 16' 00" W
14	974	Catu-BA	NE	12° 20' 60" S	38° 23' 60" W
15	976	Monte Recôncavo-BA	NE	12° 37' 88" S	38° 37' 00" W
16	977	Valença-BA	NE	13° 19' 60" S	39° 15' 00" W
17	978	Nazaré -BA	NE	12° 51' 45" S	38° 58' 55" W
18	979	Ladeira Grande-BA	NE	13° 00' 00" S	39° 01' 60" W
19	980	Barreiro-BA	NE	12° 10' 60" S	38° 23' 60" W
20	983	Chiador-MG	SE	22° 00' 00" S	43° 00' 00" W
21	985	Cuiabá-MT	CO	15° 40' 00" S	55° 32' 60" W
22	986	Nortelândia-MT	CO	14° 28' 60" S	56° 45' 00" W
23	988	Campo Verde-MT	CO	15° 39' 00" S	55° 17' 60" W
24	990	Anastácio-MS	CO	20° 34' 00" S	55° 36' 00" W
25	993	Coxim-MS	CO	18° 15' 00" S	54° 40' 00" W
26	997	Senador Canedo-GO	CO	16° 37' 60" S	49° 07' 60" W
27	999	Capinzal do Norte-MA	NE	04° 37' 00" S	44° 22' 00" W
28	1000	Porto Franco-MA	NE	06° 19' 60" S	47° 19' 60" W
29	1001	Porto Franco-MA	NE	06° 25' 60" S	47° 21' 00" W
30	1003	Porto Franco-MA	NE	06° 19' 60" S	47° 19' 60" W
31	1007	Alexânia-GO	CO	16° 10' 00" S	48° 30' 00" W
32	1009	Rio Verde-MS	CO	18° 43' 88" S	54° 47' 60" W
33	1010	Coxim-MS	CO	18° 30' 00" S	54° 45' 00" W
34	1013	Itiquira-MT	CO	17° 08' 88" S	54° 51' 00" W
35	1016	São José da Serra-MT	CO	15° 40' 00" S	55° 21' 00" W
36	1017	Cuiabá-MT	CO	15° 43' 00" S	55° 43' 60" W
37	1018	Campo Verde-MT	CO	15° 43' 00" S	55° 17' 60" W
38	1019	Primavera do Leste-MT	CO	15° 35' 60" S	54° 07' 60" W
39	1020	General Carneiro-MT	CO	15° 40' 00" S	52° 46' 60" W
40	1025	Lagoa da Confusão-TO	N	11° 34' 00" S	50° 40' 00" W
41	1026	Campo Verde-MT	CO	15° 16' 00" S	54° 55' 60" W

Continued on next page

Table 1 continued

42	1240	Belo Horizonte-MG	SE	19° 55' 17" S	43° 56' 05" W
43	1243	Abadiânia-GO	CO	16° 07' 88" S	48° 37' 60" W
44	1246	Campinorte-GO	CO	14° 22' 60" S	49° 10' 00" W
45	1250	Paraíso do Tocantins-TO	N	10° 04' 00" S	48° 49' 60" W
46	1252	Miranorte-TO	N	09° 15' 00" S	48° 34' 60" W
47	1253	Wanderlândia-TO	N	06° 52' 00" S	47° 51' 00" W
48	1254	Darcinópolis-TO	N	06° 45' 00" S	47° 49' 60" W
49	1256	Dom Eliseu-PA	N	04° 07' 60" S	47° 28' 00" W
50	1257	Nova Rosalândia-TO	N	10° 31' 00" S	49° 01' 60" W
51	1259	Porto Nacional-TO	N	10° 58' 60" S	48° 17' 60" W
52	1260	Sta Rosa do Tocantins-TO	N	11° 19' 00" S	47° 52' 00" W
53	1262	Lavrado-TO	N	12° 07' 00" S	46° 28' 60" W
54	1264	Monte Alegre-GO	CO	13° 15' 00" S	47° 08' 60" W
55	1265	São João da Aliança-GO	CO	14° 30' 00" S	47° 31' 00" W
56	1267	Planaltina-GO	CO	15° 22' 60" S	47° 31' 60" W
57	1268	Alexânia-GO	CO	16° 07' 00" S	48° 22' 00" W
58	1270	Linda Vista-GO	CO	13° 18' 00" S	49° 07' 60" W
59	1271	Porangatu-GO	CO	12° 52' 00" S	49° 07' 60" W
60	1274	Goiânia-GO	CO	16° 34' 03" S	49° 15' 54" W
61	4944	Matias Cardoso-MG	SE	15° 01' 48" S	43° 51' 04" W
62	4945	Monte azul-MG	SE	15° 14' 01" S	43° 03' 32" W
63	4946	Monte azul-MG	SE	15° 15' 12" S	43° 02' 60" W
64	4947	Pajeú-MG	SE	15° 17' 12" S	42° 54' 39" W
65	4950	Andaraí-BA	NE	12° 48' 39" S	41° 19' 30" W
66	4951	Andaraí-BA	NE	12° 48' 48" S	41° 19' 26" W
67	4952	Palmeiras-BA	NE	12° 27' 33" S	41° 29' 51" W
68	4953	Palmeiras-BA	NE	12° 27' 15" S	41° 29' 14" W
69	4954	Palmeiras-BA	NE	12° 27' 15" S	41° 29' 21" W
70	4955	Palmeiras-BA	NE	12° 27' 09" S	41° 29' 13" W
71	4956	Palmeiras-BA	NE	12° 27' 10" S	41° 29' 24" W
72	4957	Iraquara-BA	NE	12° 22' 07" S	41° 31' 00" W
73	4959	Iraquara-BA	NE	12° 22' 08" S	41° 31' 01" W
74	4960	Iraquara-BA	NE	12° 22' 09" S	41° 30' 59" W
75	4962	Utinga-BA	NE	12° 06' 11" S	41° 06' 48" W
76	4963	Morro do Chapéu-BA	NE	11° 55' 02" S	41° 08' 02" W
77	4965	Cafarnaum-BA	NE	11° 45' 25" S	41° 32' 01" W
78	4966	Oliveira dos Brejinhos-BA	NE	12° 18' 06" S	42° 37' 55" W
79	4986	Aracatu-BA	NE	14° 28' 36" S	41° 26' 41" W
80	5180	Francisco Sá-MG	SE	16° 27' 56" S	43° 25' 54" W

(TECNAL Scientific Equipments, Piracicaba, SP, Brazil) for 3 hours. Ashing was done by placing folded filter bags in crucibles in a muffle furnace at 500°C for at least 5.5 hours. N concentration was determined by Kjeldahl method (AOAC, 1996) with a Tecnal™ 0365 digestion–distillation system and CP was calculated as $N \times 6.25$. True IVDMD was determined by 48-hour ruminal fermentation at 39.5°C in a Tecnal™ *in vitro* incubator system using the procedure described by Tilley and Terry (1963), with modification by Goering and Van Soest (1970). Hemicellulose concentration (Hemic) was estimated by subtracting ADF from NDF and Cellulose (CELLU) by subtracting LIG from ADF.

Statistical Analysis

Univariate analysis was performed through the F test to detect potential differences among sites of collection as well as similarity clusters for each quality trait. Multivariate analysis was used to examine the genetic variability of the accessions. A hierarchical clustering analysis was done to sort out groups of similarity based on Ward's minimum variance method and their genetic dissimilarity were estimated by the square root of the generalized Mahalanobis D^2 distances (Alvares et al, 2012; Bapurao et al, 2018). Cluster analysis allows classifying accessions into homogeneous groups based on their quality-traits values. Accessions within the same group are more similar and homogeneous while accessions among different groups are more divergent and heterogeneous.

A principal components analysis (PCA) was performed to discriminate among accessions and group them. The goal of PCA is to provide a reduced dimension model that would indicate differences among groups of similarity. It also contributes to a better understanding of the variables by describing how much of the total variance was explained by each one of them. Also, PCA analysis allows graphical visualization of the variables, identification of similar and divergent accessions, and highlights the variables that contribute most to the differentiation of accessions (Philippeau and Philippeau, 1986). All statistical analyses were done with the R software version 3.6.2 programme (R Core Team, 2019).

The phenotypic diversity among accessions was estimated by the index of Shannon and Weaver (1949) corrected by the logarithm of the number of classes and Simpson (1949): Shannon and Weaver corrected: $J = H' / \ln S = -\sum p_i \cdot \ln p_i / \ln S$; and Simpson: $D = 1 / \sum p_i^2$, where J is the Shannon corrected index, H' is the richness and evenness Shannon index, p_i is the proportion of the class i, and $\ln S$ the natural logarithm of the total number of classes. Both indices are used to express the concepts of richness, meaning the number of different accessions in a sampling effort, as well as evenness, meaning the degree of equality in the abundance of individuals, or the relative uniformity of their distribution across groups of accessions (Meng et al, 1999). These indices are used to estimate diversity within populations (Kosman and Leonard,

2007) and range from zero to one, where one represents high genetic diversity and zero no genetic diversity. They correspond to the probability of two individuals randomly selected from a group to exhibit similar characteristics (Carvalho and Quesenberry, 2009).

Results

The average concentration of CP, IVDMD, NDF, ADF, LIG, HEMIC and CELLU were $220.6 \pm 20.2\text{g/kg}$, $560 \pm 56.0\text{g/kg}$, $516.8 \pm 44.1\text{g/kg}$, $368 \pm 32.9\text{g/kg}$, $69.4 \pm 9.4\text{g/kg}$, $148.8 \pm 31.5\text{g/kg}$ and $298.6 \pm 32.9\text{g/kg}$, respectively (Table 2).

Means of IVDMD, ADF and CELLU were significantly different for site of collection (Brazilian states, Table 3). Accessions from BA, GO, PA and TO had higher IVDMD concentrations. PA (only one sample) and TO states, had the highest IVDMD concentration, 653.4g/kg and $590.6 \pm 18.5\text{g/kg}$, respectively, and were significantly different from those for MS and MT states. Accessions from GO, MA, PA, and TO had the lowest concentrations of ADF and CELLU, with $368 \pm 32.9\text{g/kg}$ and $298.6\text{g} \pm 32.9\text{g/kg}$, respectively. Lower fibre concentrations were also found for PA (only one accession) and TO, with 341.4g/kg and $339.8 \pm 28.3\text{g/kg}$ of ADF and 264.5g/kg and $272.1 \pm 23.2\text{g/kg}$ of CELLU, respectively (Table 3).

The PCA of the 80 accessions resulted in five principal components explaining all variations (Table 4). The first two principal components accounted for 73% of the total variance among all accessions. The first PC was responsible for 55% and the second for 18% of the variation. Variables that are correlated with PC1 and PC2 are the most important in explaining the variability in the data set. The correlation between each variable and the PC was used as the coordinate in a circle of radius equals to one, and represents the intensity in which the variable contribute to PC1 and PC2 (Figure 1). Traits ADF, NDF, IVDMD and CP contributed most to PC1, with values ranging from 0.73 to 0.90, while only LIG had an important contribution to PC2, with a value of 0.76. Figure 1 displays the correlation circle of the PCA. Positively correlated variables point to the same side of the plot and negatively correlated variables point to opposite sides of the graph.

The Kelley–Gardner–Sutcliffe penalty function for hierarchical cluster trees was used to define the number of groups (Kelley et al, 1996). The minimum of this function defines the group size. In this study, clustering of the 80 accessions resulted in four clusters, which means four diversity classes of forage nutritive values, considering CP, IVDMD, NDF, ADF, and LIG concentrations (Figure 2). All traits had high and similar diversity values from Simpson's that ranged from 0.771 to 0.781 and Shannon–Weaver's indices that ranged from 0.806 to 0.817 (Table 5).

Discussion

In Australia, 'Seca' and 'Fitzroy' *S. scabra* cultivars have been released with high dry matter yields, ade-

Table 2. Mean $\pm$ standard deviation (SD), minimum and maximum values (Range) in g/kg, site of collection (state) mean square (MSs), and p-value of five quality traits from 80 *Stylosanthes scabra* Vogel germplasm accessions. *, ** significant differences among states at 0.05 and 0.01 α levels, respectively.

Trait	Statistics			
	Mean $\pm$ SD	Range	MSs	p-value (Pr>F)
Crude Protein	220.6 $\pm$ 20.2	185.1–279.2	6.748	0.2003
<i>In Vitro</i> Dry Matter Digestibility	560.0 $\pm$ 56.0	427.9–718.8	71.550*	0.0127
Neutral Detergent Fibre	516.8 $\pm$ 44.1	419.3–630.1	21.640	0.3550
Acid Detergent Fibre	368.0 $\pm$ 32.9	288.2–456.4	31.045**	0.0014
Lignin	69.4 $\pm$ 9.4	45.7–91.6	1.243	0.186
Hemicellulose	148.8 $\pm$ 31.5	103.7–264.3	13.031	0.229
Cellulose	298.6 $\pm$ 29.9	229.9–395.3	30.034**	0.00016

Table 3. Seven quality traits, estimated from 80 *Stylosanthes scabra* Vogel germplasm accessions by Brazil source states. Given are the number of accessions per state and values in g/kg for means $\pm$ standard deviation (Mean $\pm$ SD), minimum and maximum values (Min, Max). Brazilian states: BA, Bahia; GO, Goiás; MA, Maranhão; MG, Minas Gerais; MT, Mato Grosso; MTS, Mato Grosso do Sul; PA, Pará; RJ, Rio de Janeiro; TO, Tocantins. CP, Crude Protein; IVDMD, *In Vitro* Dry Matter Digestibility; NDF, Neutral Detergent Fibre; ADF, Acid Detergent Fibre; LIG, Lignin; HEMIC, Hemicellulose; CELLU, Cellulose. Mean values for traits followed by the same superscript letter are not significantly different, based on LSD - t student at 0.05 α level.

Trait	State	BA	GO	MA	MG	MS	MT	PA	RJ	TO
CP	Nº	32	11	4	7	4	11	1	1	9
	Mean	221.8	233.6	226.7	218.5	241.2	226.1	209.3	195.9	239.8
	$\pm$ SD	27.7	12.9	10.5	17.0	6.5	19.9	-	-	15.6
	Min	185.1	218.3	213.6	196.8	235.2	185.3	209.3	195.9	215.6
	Max	279.2	257.0	238.7	240.8	249.6	250.2	209.3	195.9	265.5
IVDMD	Mean	572.1 ^{ab}	563.0 ^{ab}	549.5 ^{abc}	558.7 ^{abc}	520.0 ^{bc}	509.8 ^c	653.4 ^a	533.0 ^{abc}	590.6 ^a
	$\pm$ SD	55.4	55.1	23.0	60.3	73.9	47.3	-	-	18.5
	Min	476.0	467.7	517.9	479.4	427.9	431.4	653.4	533.7	563.9
	Max	718.8	658.9	567.4	651.1	587.9	587.7	653.4	533.7	611.4
	Mean	511.1	515.3	518.6	539.1	502.6	541.3	470.6	541.7	499.8
NDF	$\pm$ SD	39.3	52.5	46.0	58.0	21.6	38.6	-	-	47.0
	Min	419.3	442.6	459.9	475.8	488.2	485.7	470.6	541.7	445.8
	Max	627.2	624.2	572.3	630.1	534.7	594.9	470.6	541.7	597.7
	Mean	370.3 ^{ab}	357.9 ^{bc}	336.0 ^c	390.0 ^a	366.6 ^{abc}	390.3 ^a	341.4 ^{bc}	420.2 ^a	339.8 ^c
	$\pm$ SD	28.8	23.3	24.4	36.1	11.8	36.5	-	-	28.3
ADF	Min	371.6	368.9	335.0	392.3	364.1	385.0	341.4	420.2	346.2
	Max	382.3	371.7	345.4	412.4	372.9	411.7	341.4	420.2	364.8
	Mean	69.2	66.5	76.2	76.4	65.0	67.2	76.9	82.0	67.7
	$\pm$ SD	9.6	8.2	13.3	6.8	5.1	9.4	-	-	9.5
	Min	49.9	55.8	62.3	68.7	59.5	45.7	76.9	82.0	58.5
LIG	Max	91.6	85.9	91.4	87.5	70.1	81.1	76.9	82.0	86.4
	Mean	140.7	157.3	182.6	149.1	136.1	151.0	129.2	121.4	160.0
	$\pm$ SD	25.7	41.3	37.9	34.2	21.3	20.2	-	-	41.4
	Min	103.7	119.2	152.5	119.5	113.3	130.3	129.2	121.4	128.4
	Max	221.1	253.6	234.1	214.9	164.8	189.9	129.2	121.4	264.3
HEMIC	Mean	301.2 ^{ab}	291.4 ^{ab}	259.9 ^c	313.5 ^a	301.5 ^{ab}	323.1 ^a	264.5 ^{bc}	338.2 ^a	272 ^{bc}
	$\pm$ SD	22.9	23.2	16.1	30.7	11.9	37.3	-	-	23.2
	Min	234.7	250.0	239.2	273.6	286.1	284.3	264.5	338.2	230.0
	Max	363.9	318.7	275.3	359.4	313.3	395.3	264.5	338.2	299.7
	Max	363.9	318.7	275.3	359.4	313.3	395.3	264.5	338.2	299.7

quate nutritive values, drought tolerance and persistence (Edye *et al*, 1998). In Nigeria, Akinlade *et al* (2008) found CP concentrations ranging from 147g/kg to 151g/kg for four *S. scabra* cultivars. In

Argentina, Ciotti *et al* (1999) evaluated 33 accessions of 6 different species of *Stylosanthes* in which 4 *S. scabra* accessions had CP mean values ranging from 78g/kg to 134g/kg. In Brazil, CP from *Stylosanthes* commercial

Table 4. Principal component analysis (PCA) of the 80 accessions of *Stylosanthes scabra* Vogel. CP, Crude Protein; IVDMD, *In Vitro* Dry Matter Digestibility; NDF, Neutral Detergent Fibre; ADF, Acid Detergent Fibre; LIG, Lignin.

PCA	PC1	PC2	PC3	PC4	PC5
Eigen values	2,77	0,89	0,67	0,43	0,24
Proportion of variance (%)	55,48	17,77	13,41	8,57	4,78
Cumulative variance (%)	55,48	73,24	86,65	95,22	100,00
CP	0,73	-0,27	0,51	-0,37	-0,08
IVDMD	0,74	-0,47	0,05	0,47	-0,08
NDF	-0,77	-0,09	0,55	0,18	0,24
ADF	-0,90	-0,07	0,16	0,05	-0,40
LIG	-0,54	-0,76	-0,29	-0,19	0,09

Table 5. Simpson and Shannon–Weaver diversity indices for *Stylosanthes scabra* Vogel.

Trait	Simpson	Shannon–Weaver
Crude Protein (CP)	0.781	0.813
<i>In Vitro</i> Dry Matter Digestibility (IVDMD)	0.771	0.806
Neutral Detergent Fibre (NDF)	0.779	0.812
Acid Detergent Fibre (ADF)	0.774	0.808
Lignin (LIG)	0.779	0.817

cultivars from different species or a mixture of species (*S. guianensis* cv. Mineirão, *S. macrocephala* cv. Pioneiro, and a mixture of *S. capitata* and *S. macrocephala* cv.

Campo Grande) are reported with concentrations ranging from 98 to 210g/kg (Braga et al, 2020). The mean value obtained in this study for *S. scabra* was 220g/kg, higher than those from Argentina and higher or similar to other studies in Brazil. This CP high value reflects the species' potential to be used as a forage in animal production systems in the tropics.

The *S. scabra* NDF and ADF values obtained in this research are similar to the ones reported for 71 accessions from five *Stylosanthes* species collected in Brazil (Santana, 2010): *S. angustifolia* with 540g/kg of NDF and 378g/kg of ADF; *S. capitata* with 490g/kg of NDF and 330g/kg of ADF; *S. macrocephala* 545g/kg of NDF and 394g/kg of ADF; *S. pilosa* with 531g/kg of NDF and 394g/kg of ADF; and *S. viscosa* with 553g/kg of NDF and 390g/kg of ADF in average. The average fibre concentration of *S. scabra* in this study was 517g/kg for NDF and 368g/kg for ADF, which indicate that the fibre content among different *Stylosanthes* species is similar. This fibre characteristics together with the high CP content indicate that *S. scabra* species may be used, as other *Stylosanthes* species, as an important forage for seasonally dry tropical and subtropical environments in Brazil.

There were significant differences among sites of collection for IVDMD, ADF, and CELLU, pointing to the existence of phenotypic variability among accessions (Table 2). The range for IVDMD, ADF and CELLU were 291g/kg, 168g/kg and 165g/kg, respectively. This indicates that there is potential genetic diversity among genotypes for IVDMD, ADF and CELLU to be successfully exploited in a breeding programme. Differences among genotypes from different states were not significant for CP, NDF, LIG, and HEMIC. Thus, genetic gains from selection might be less effective when selecting for high CP and HEMIC, as well as low NDF and LIG from this collection.

The negative correlation between digestibility and fibre concentrations in forage species is well known because the carbohydrates in the NDF and ADF portions of plant tissues are mostly cell walls indigestible to ruminants. Thus, the lower ADF and CELLU concentrations from PA and TO indicate that those accessions may produce segregant populations when used in breeding programmes for higher nutritive quality forage. This is only an indication, additional studies should be carried out since higher IVDMD and lower fibre contents may not be due only to genetic effects but also management practices, plant age and other environmental effects.

The goal of PCA is to provide a reduced dimension model that would indicate measured differences among groups. It also can contribute to a better understanding of the set of variables by describing how much of the total variance is explained by each one. The circle of correlations graphic illustrates the direction as well as the intensity of the variable vectors in the first two components (Figure 1). Note that CP, IVDMD, NDF, ADF vectors have closer inclinations along PC1, with CP and

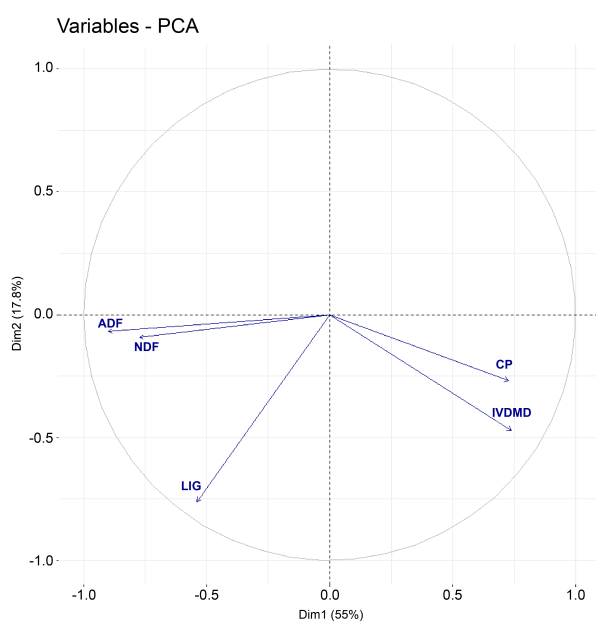


Figure 1. Correlation circle illustrating the direction and intensity of the five forage quality variables used in the principal component analysis. CP, Crude Protein; IVDMD, *In Vitro* Dry Matter Digestibility; NDF, Neutral Detergent Fibre; ADF, Acid Detergent Fibre; LIG, Lignin.

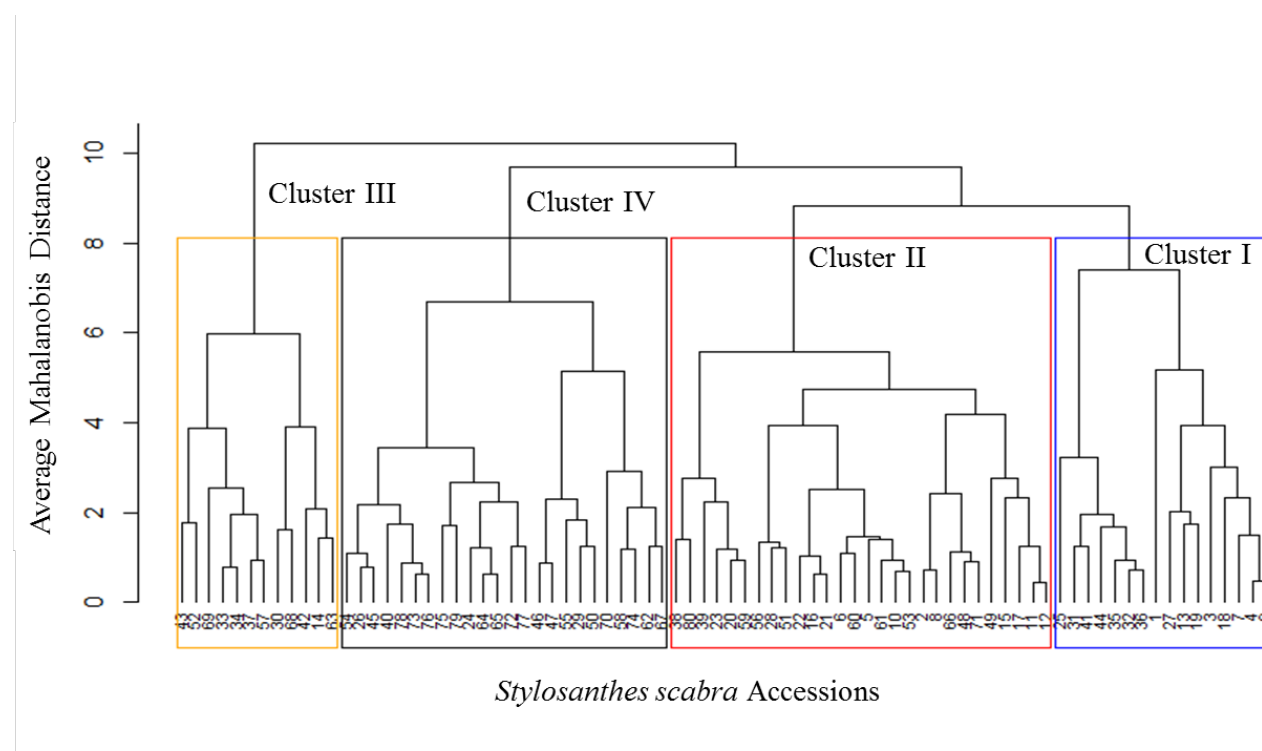


Figure 2. Dendrogram illustrating the distribution of 80 accessions of *Stylosanthes scabra* Vogel in four clusters based on Mahalanobis distance. The accessions are numbered as in [Table 1](#).

IVDMD vectors pointing to the same orientation, as are NDF and ADF but in opposite directions. The IVDMD and ADF concentrations are the closest to the circle of correlations, indicating the better representation of the traits on the definition of PC1. Yet, LIG was the only trait with a better representation on PC2. Variables close to the centre of the circle are less important for the first component. In brief, there was a positive correlation between CP and IVDMD and between NDF and ADF, as well as negative correlations among the former traits with the latter ones. ADF, IVDMD and LIG were the traits with the best representations on the first two principal components.

In this study, 80 accessions resulted in four clusters, which means four diversity classes of forage nutritive values, considering CP, IVDMD, NDF, ADF, and LIG concentrations ([Figure 2](#)). Concentrations of HEMIC and CELLU were not considered in the analysis since they are highly correlated to NDF and ADF concentrations, respectively, from which they are directly derived ([Table 4](#)). The four Mahalanobis distance clusters were plotted in the PCA graphic, each in a 0.95 probability level ellipsis, to get a better view of their distribution ([Figure 3](#)). Scatter plots of the principal component scores were convergent with the clusters' ellipsis, allowing the visualization and identification of locations with higher concentrations of a given trait in the forage.

Four out of the five original variables had high contributions to the first principal component. This first component is a measure of forage quality when IVDMD and CP increase. Also, it is a measure of lack

of quality when the fibre components NDF and ADF increase. Furthermore, the first principal component correlates most strongly with the ADF ($r = 0.90$). Thus, this first principal component may be considered primarily a measure of the ADF concentration. This implies that accessions with higher values of ADF tend to have a lower quality of forage. The second principal component increases with the decrease of LIG concentration. This component can be viewed as a measure of forage quality as LIG concentration decreases. PCA analysis did separate the accessions into two dimensions, even though the traits are quantitative with a scatter distribution along PC1 and PC2. All five quality characteristics had high loading values in the first two principal components, ranging from 0.73 to 0.90, indicating their importance as *S. scabra* descriptors.

Cluster II and IV with 28 and 24 accessions, respectively, were the largest, representing together 65% of the total accessions. Cluster I with 16 accessions and Cluster III with 12 accessions represented the remaining 35% of the total number of accessions ([Table 6](#)). The analyses of variance among clusters for each trait are presented in [Table 7](#). There were highly significant differences among clusters for CP, IVDMD and ADF, indicating substantial genetic variability. Cluster IV included accessions with the highest CP and IVDMD concentrations and the lowest ADF concentration, representing the group of accessions with the higher forage quality, and may be used for hybridization in breeding programmes. Clusters I and II had the lowest CP and IVDMD, as well as the highest ADF

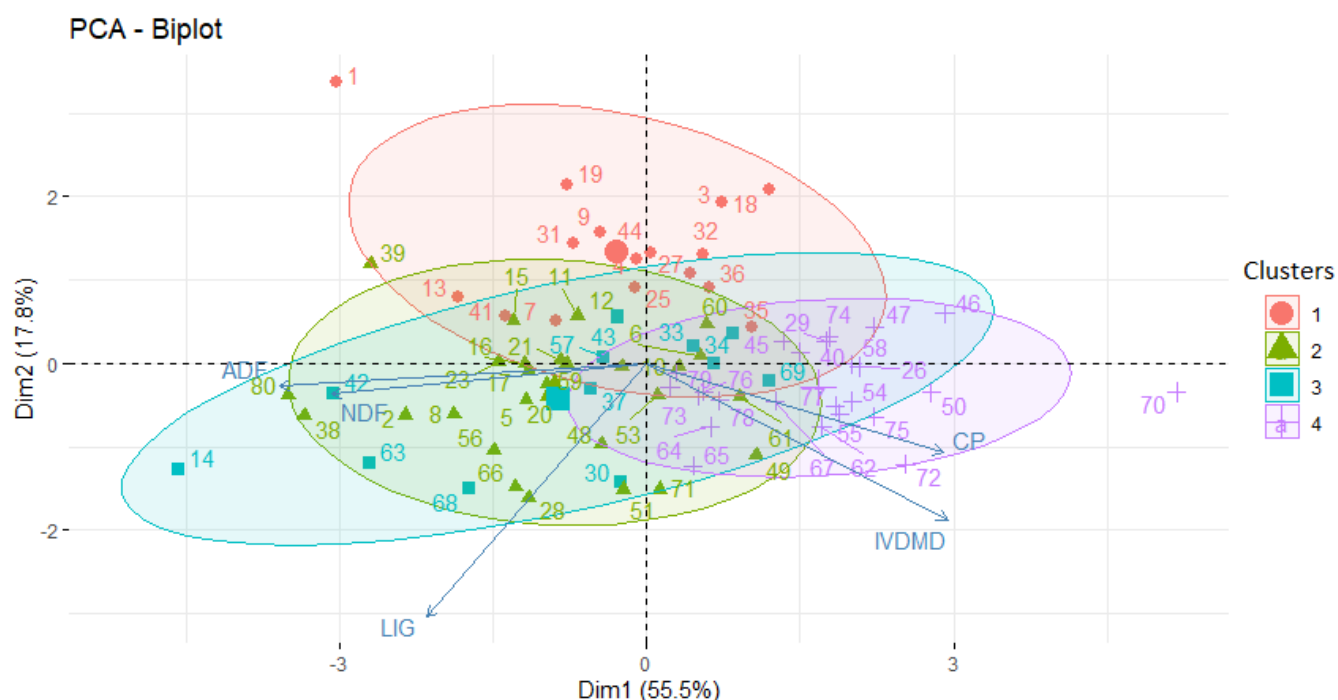


Figure 3. Dispersion of 80 *Stylosanthes scabra* Vogel accessions in relation to the first two components and four Mahalanobis similarity clusters. The accessions are numbered as in Table 1 and grouped in four clusters as in Table 6.

concentrations, representing the lowest nutritive value cluster. Cluster III had intermediate values for all traits and its CP concentration was significantly different from all other clusters.

Formation of similarity groups and respective estimation of their divergence is useful when choosing accessions to be used as progenitors for superior hybrid combinations in breeding programmes. Divergence estimates by Mahalanobis D distances are presented in Table 8. The intra-group distances were very similar for all four clusters, ranging from 3.15 to 3.37, and were consistently lower than the inter-cluster distances, which ranged from 6.92 to 12.47, as expected. These distances clearly suggest a great deal of genetic similarity within and genetic divergence among clusters. The shortest inter-cluster distance was between Cluster II, indicating lower divergence. The highest inter-group distance was 12.46 between Clusters I and III, followed by 11.99 between Clusters III and IV, and 11.98 between Clusters I and IV. High divergences among groups were consistent with the ANOVA results where Cluster IV was significantly different from Clusters I and III, for CP, IVDMD and ADF. In a breeding programme, crosses between accessions with maximum divergence may give rise to genetically diverse lines and the potential production of heterotic transgressive segregants. A great deal of variation in subsequent generations is expected from crosses among plants of maximum inter-class distances clusters. This may not be true between low-divergent clusters as Clusters I and II.

The pattern of distribution of accessions in different clusters did not reflect the geographical diversity of the site of collection (states). Accessions from each state

were consistently distributed among the four clusters of forage quality (Figure 4). Cluster I presented accessions from five states, Cluster II from eight, Cluster III from seven, and Cluster IV from six, all out of a total of nine states. Thus, the observed traits diversity was somewhat similar among the different collection sites, leading to a lack of effect of the source locations on the four quality groups definition considering this germplasm collection.

Simpson's and Shannon-Weaver's indices expressed the diversity among accessions within each population,

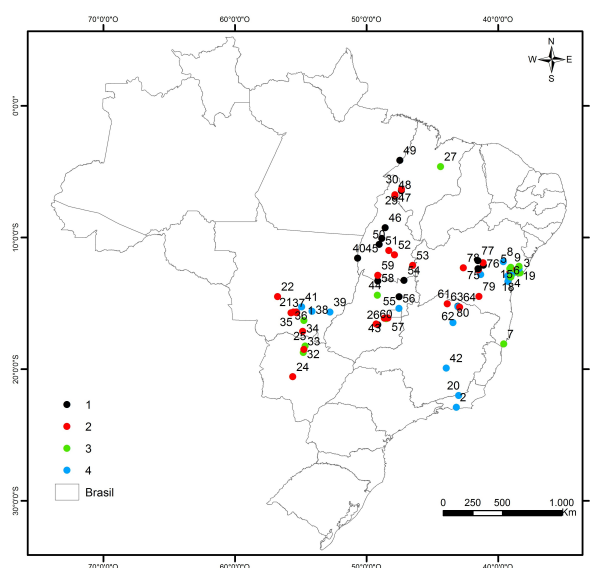


Figure 4. Geographic distribution of collection sites of the 80 *Stylosanthes scabra* Vogel accessions in Brazil, coloured according to the clusters in which they were grouped.

Table 6. Distribution of 80 *Stylosanthes scabra* Vogel accessions in four clusters based on the forage quality variables. Embrapa Cerrados, Planaltina, DF, 2020.

Cluster	Nº of Accessions	Accessions ID
I	16	1, 3, 4, 7, 9, 13, 18, 19, 25, 27, 31, 32, 35, 36, 41, 44
II	28	2, 5, 6, 8, 10, 11, 12, 15, 16, 17, 20, 21, 22, 23, 28, 38, 39, 48, 49, 51, 53, 56, 59, 60, 61, 66, 71, 80
III	12	14, 3033, 34, 37, 42, 43, 52, 57, 63, 68, 69
IV	24	24, 26, 29, 40, 45, 46, 47, 50, 54, 55, 58, 62, 64, 65, 67, 70, 72, 73, 74, 75, 76, 77, 78, 79

Table 7. Mean concentrations $\pm$ standard deviations of CP, IVDMD, NDF, ADF, and LIG of *Stylosanthes scabra* Vogel accessions distributed in four clusters. ** Significant differences among clusters at 0.01 α level. Means followed by the same subscript letter within the same column are not different, LSD - t student at 0.05 α level. CP, Crude Protein; IVDMD, *In Vitro* Dry Matter Digestibility; NDF, Neutral Detergent Fibre; ADF, Acid Detergent Fibre; LIG, Lignin.

	CP (%)	IVDMD (%)	NDF (%)	ADF (%)	LIG (%)
Cluster I	21,58 $\pm$ 2.21 _c	50,35 $\pm$ 4.27 _c	50,98 $\pm$ 3.32	36,73 $\pm$ 2.64 _a	6,13 $\pm$ 0.72
Cluster II	21,25 $\pm$ 1.35 _c	54,81 $\pm$ 4.40 _b	52,06 $\pm$ 2.99	38,36 $\pm$ 2.77 _a	7,63 $\pm$ 0.63
Cluster III	22,97 $\pm$ 1.74 _b	55,96 $\pm$ 3.88 _b	58,36 $\pm$ 3.52	37,65 $\pm$ 3.66 _a	7,19 $\pm$ 1.24
Cluster IV	24,82 $\pm$ 1.41 _a	61,18 $\pm$ 3.72 _a	48,37 $\pm$ 2.51	34,60 $\pm$ 2.98 _b	6,55 $\pm$ 0.54
Mean	22.65 $\pm$ 1.72**	56,00 $\pm$ 4.12**	51.68 $\pm$ 4.37n.s	36.80 $\pm$ 3.14**	6,94 $\pm$ 0.95n.s.
Range	18.53–27.92	42.79–71.88	44.13–52.41	33.18–40.23	4.57–7.56

Table 8. Mean intra- and inter-cluster Mahalanobis (D) distances of 80 accessions of *Stylosanthes scabra* Vogel. Embrapa Cerrados, Planaltina, DF, 2020.

Cluster	I	II	III	IV
I	3.22			
II	6.92	3.15		
III	12.47	9.71	3.37	
IV	11.98	9.79	11.99	3.18

in this case, each different Brazilian state where collecting was done. High values for both indices suggest a significant amount of genetic variation for forage quality traits.

Conclusions

There is considerable genetic diversity for forage nutritive values among the *S. scabra* accessions from Embrapa Cerrado's germplasm bank. This variability was reflected across geographic areas in Brazil for *in vitro* digestibility, acid detergent fibre, and cellulose concentrations.

Independent of the site of collection (Brazilian states), 24 high-quality *S. scabra* accessions are promising parents for breeding due to their higher digestibility and protein content, as well as lower lignin concentrations.

S. scabra accessions from Embrapa Cerrado's germplasm bank presented significant genetic diversity that may be useful for developing new high-quality improved forage populations in a breeding programme.

Author contributions

All authors contributed to the study conception and design. Fabiana Karla de Araújo Américo, Marcelo Ayres

Carvalho, Allan Kardec Braga Ramos and Claudio Takao Karia did all genetic material preparation, experiment implementation and conduction, and data collection and organization. Fabiana Karla de Araújo Américo and Carlos Eduardo Lazarini da Fonseca did the wet lab analysis. Juaci Vitória Malaquias, Marcelo Ayres Carvalho and Carlos Eduardo Lazarini da Fonseca did the data analysis. Fabiana Karla de Araújo Américo, Marcelo Ayres Carvalho and Carlos Eduardo Lazarini da Fonseca wrote the first draft of the manuscript, and all authors commented on the different versions of the manuscript. Marina de Fátima Vilela did the geographical information plots. Gustavo José Braga was responsible for reviewing the manuscript. All authors read and approved the final manuscript.

Conflict of interest statement

All authors have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Funding

This work was partially funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES (Coordination for the Improvement of Higher Education Personnel, a Federal Agency) which awarded a scholarship to the first author. This work was part of her dissertation in partial fulfilment of the requirements of Doctor of Science (DSc) in Plant Genetic Resources from the Universidade Federal de Feira de Santana.

References

- Akinlade, J. A., Farinu, G. O., Agboola, O. O., Akingbade, A. A., Ojebiyi, O. O., and Aderinola, O. A. (2008). Research note: Nutritive value of four accessions of *Stylosanthes scabra* in the derived savanna zone of Nigeria. *Tropical Grasslands* 42(2). url: https://www.researchgate.net/publication/268380547_Research_note_Nutritive_value_of_four_accessions_of_Stylosanthes_scabra_in_the_derived_savanna_zone_of_Nigeria.
- Alvares, R. C., Reis, E. F. D., and Pinto, J. F. N. (2012). Genetic divergence in pepper genotypes from southwest Goiás. *Ciência e Agrotecnologia* 36(5), 498–506. doi: <https://doi.org/10.1590/S1413-70542012000500002>
- AOAC (1996). Official Methods of Analysis of the Association of Official Analytical Chemists (Arlington: AOAC International).
- Bapurao, A. B., Kumar, S., Suresh, B. G., and Kuma, A. (2018). Genetic diversity studies in chickpea (*Cicer arietinum* L.) germplasm. *Journal of Food Legumes* 31(4), 261–264. doi: <https://doi.org/10.4238/vol9-3gmr862>
- Boddey, R. M., Casagrande, R. M., Homem, B. G. C., and Alves, B. J. R. (2020). Forage legumes in grass pastures in tropical Brazil and likely impacts on greenhouse gas emissions: A review. *Grass and Forage Science* 75, 357–371. doi: <https://doi.org/10.1111/gfs.12498>
- Boldt, A. S. (2011). Diversidade genética, adaptabilidade e estabilidade de genótipos de soja no Mato Grosso. Ph.D. thesis, Universidade Federal de Viçosa, Viçosa, Brazil.
- Braga, G., Ramos, J., Carvalho, A. K. B., Fernandes, M. A., F. Fonseca, D. D., Fernandes, C. E. L., and D, C. (2020). Ganho de peso de bovinos nelore em pastagens consorciadas com replantio do estilósantes BRS Bela. *Embrapa Cerrados, Boletim de Pesquisa e Desenvolvimento*. url: <https://ainfo.cnptia.embrapa.br/digital/bitstream/item/222980/1/Bolpd-368-web.pdf>.
- Cameron, D. (1983). To breed or not to breed. In *Genetic resources of forage plants*, ed. McIvor, J. and Bray, R., (Melbourne: CSIRO), 237–250.
- Cameron, D. F., Chakraborty, S., and S (2004). Forage potential of *Stylosanthes* in different production systems. In *High-Yielding Anthracnose-Resistant Stylosanthes for Agricultural Systems*, ed. Chakraborty, S. (ACIAR), 27–38.
- Carvalho, G. G. P. and Pires, A. J. V. (2008). Leguminosas tropicais herbáceas em associação com pastagens. *Arch. Zootec* 57, 103–113. url: https://edisciplinas.usp.br/pluginfile.php/4980430/mod_resource/content/1/Leguminosas_Carvalho.pdf
- Carvalho, J. M., Barreto, R. F., Prado, R. D. M., Habermann, E., Martinez, C. A., and Branco, R. B. F. (2020). Elevated [CO₂] and warming increase the macronutrient use efficiency and biomass of *Stylosanthes capitata* vogel under field conditions. *J. Agron. Crop Sci* 206, 597–606. doi: <https://doi.org/10.1111/jac.12398>
- Carvalho, M. A. and Quesenberry, K. H. (2009). Morphological characterization of the USA *Arachis pintoi* Krap. and Greg. collection. *Plant Systematics and Evolution* 277(1-2), 1–11. doi: <https://doi.org/10.1007/s00606-008-0089-9>
- Ciotti, E. M., Tomei, C. E., and Castelan, M. E. (1999). The adaptation and production of some *Stylosanthes* species in Corrientes, Argentina. *Tropical Grasslands* (33), 165–169. url: https://tropicalgrasslands.info/public/journals/4/Historic/Tropical%20Grasslands%20Journal%20archive/PDFs/Vol_33_1999/Vol_33_03_99_pp165_169.pdf.
- Costa, N. M. (2006). Revisão do Gênero de *Stylosanthes* Sw. Ph.D. thesis, Universidade Técnica de Lisboa, Instituto Superior de Agronomia, Lisboa. Portugal.
- Cruz, C. D., Ferreira, F. M., and Pessoni, L. A. (2011). Biometria aplicada ao estudo da diversidade genética (Visconde do Rio Branco: Suprema).
- Edye, L. A., Hall, T. J., Clem, R. L., Graham, T. W. G., Messer, B., and Rebgets, R. H. (1998). Sward evaluation of eleven “*Stylosanthes seabra*” accessions and *S. scabra* cv. Seca at five subtropical sites. *Tropical Grasslands* (32), 243–251. url: https://www.tropicalgrasslands.info/public/journals/4/Historic/Tropical%20Grasslands%20Journal%20archive/PDFs/Vol_32_1998/Vol_32_04_98_pp243_251.pdf
- Epifanio, P. S., Costa, K. A., Severiano, E. C., Simon, G. A., and Silva, V. R. (2020). Nitrogen nutrition and changes in the chemical attributes of the soil for cultivars of *Brachiaria brizantha* intercropped with *Stylosanthes* in different forage systems. *Archives of Agronomy and Soil Science* 66(8), 1154–1169. doi: <https://doi.org/10.1080/03650340.2019.1658867>
- Goering, H. K. and Van Soest, P. J. (1970). Forage fibre analysis (apparatus, reagents, procedures, and some applications). *Agric. Res. Serv.* url: <https://naldc.nal.usda.gov/download/CAT87209099/PDF>.
- Hoffmann, A., Moraes, E. H. B. K., Mousquer, C. J., Simioni, T. A., Gomer, F. J., Ferreira, V. B., and Silva, H. M. (2014). Produção de bovinos de corte no sistema de pasto-suplemento no período seco. *Nativa* 2(2), 119–130. doi: <https://doi.org/10.14583/2318-7670.v02n02a10>
- IBGE (2017). Censo agropecuário 2017 - Resultados preliminares. url: <https://censos.ibge.gov.br/agro/2017/templates/censo-agro/resultadosagro/estabelecimentos.html>.
- Kelley, L. A., Gardner, S. P., and Sutcliffe, M. J. (1996). An automated approach for clustering an ensemble of NMR-derived protein structures into conformationally related subfamilies. *Protein Engineering, Design and Selection* 9(11), 1063–1065. doi: <https://doi.org/10.1093/protein/9.11.1063>
- Komarek, A. R., Robertson, J. B., and Van Soest, P. J. (1994). A comparison of methods for determining

- ADF using the filter bag technique versus conventional filtration. *J. Dairy Sci* (77), 114–114.
- Komarek, A. R. and Sirois, P. (1993). An improved filtering technique for the analysis of neutral detergent fiber and acid detergent fiber utilizing the filter bag technique. *J Anim Sci* (71), 824–829.
- Kosman, E. and Leonard, K. J. (2007). Conceptual analysis of methods applied to assessment of diversity within and distance between populations with asexual or mixed mode of reproduction. *New Phytologist* 174(3), 683–696. doi: <https://doi.org/10.1111/j.1469-8137.2007.02031.x>
- Kretschmer, A. E. (1988). Consideraciones sobre factores que afectan la persistencia de leguminosas forrajeras tropicales. *Pasturas Tropicales* (10), 28–33. url: https://www.tropicalgrasslands.info/public/journals/4/Elements/DOCUMENTS/1988-vol10-rev1-2-3/Vol10_rev1.88_art7.pdf.
- Marques, A., Moraes, L., Santos, M. A., Costa, I., Costa, L., Nunes, T., Melo, N. F., Simon, M. F., Leitch, A. R., Almeida, C., and Souza, G. (2018). Origin and parental genome characterization of the allotetraploid *Stylosanthes scabra* Vogel (Papilionoideae, Leguminosae), an important legume pasture crop. *Annals of Botany* (122), 1143–1159. doi: <https://doi.org/10.1093/aob/mcy113>
- Meng, E. C., Smale, M., Ruifa, H., Brennan, J. P., and Godden, D. P. (1999). Measurement of Crop Genetic Diversity in Economic Analysis. 2016-2559.
- Mertens, D. R. (2002). Gravimetric determination of amylase-treated neutral detergent fibre in feeds with refluxing beakers or crucibles: collaborative study. *J. Assoc. Off. Assoc. Chem. Int* (85), 1217–1240. url: <https://pubmed.ncbi.nlm.nih.gov/12477183/>
- Moreira, J. A. S., Fagundes, J. L., Mistura, C., Lemos, N. L. S., Moreira, J. N., Backes, A. A., Morais, J. A. S., Oliveira, V. S., and Moreira, A. L. (2015). url: <http://doi.org/10.5433/1679-0359.2015v36n1p391>.
- Neres, M. A., Castagnara, D. D., Silva, F. B., Oliveira, P. S. R., Mesquita, E. E., Bernardi, T. C., Guaranti, A. J., and Vogt, A. S. L. (2012). Características produtivas, estruturais e bromatológicas dos capins Tifton 85 e Piatã e do feijão-guandu cv. Super N, em cultivo singular ou em associação. *Ciência Rural* (42), 862–869. url: <https://www.scielo.br/j/cr/a/8jzd4ddxNWz8Rmn76nLtSmp/?format=pdf&lang=pt>.
- Peel, M. C., Finlayson, B. L., and McMahon, T. A. (2007). Updated world map of the Köppen-Geiger climate classification. *Hydrology and Earth System Sciences Discussions* (4), 439–473. doi: <https://doi.org/10.5194/hess-11-1633-2007>
- Pereira, R. C., Ribeiro, K. G., Pereira, O. G., Silva, J. L., Santos, J. M., and Rigueira, J. P. S. (2011). Produtividade e composição bromatológica de *Brachiaria* spp. *Alto Vale do Jequitinhonha. Ciência e Agrotecnologia* 524-530. doi: <https://doi.org/10.1590/S1413-70542011005000005>
- Philippeau, G. and Philippeau, G. (1986). Comment interpréter les résultats d'une analyse en composantes principales. (Institut technique des céréales et des fourrages (ITCF)).
- R Core Team (2019). R: A language and environment for statistical computing (Vienna, Austria: R Foundation for Statistical Computing). url: <https://www.R-project.org/>.
- Resende, M. R. S., Valle, C. B., and Jank, L. (2008). Melhoramento de Forrageiras Tropicais. 1 ed. Embrapa Gado de Corte (Campo Grande, MS.) url: https://www.researchgate.net/publication/200078957_Melhoramento_genetico_de_leguminosas_forrageiras.
- Rocha, J. E. S. (2014). Melhoramento vegetal e recursos genéticos forrageiros. *Embrapa Caprinos e Ovinos, Sobral*. url: <https://www.infoteca.cnptia.embrapa.br/infoteca/bitstream/doc/1007405/1/CNPC2014Melhoramento.pdf>.
- Santana, A. S. (2010). Resgate, caracterização morfológica e avaliação bromatológica de acessos de *Stylosanthes* sp. do Semiárido Baiano.
- Shannon, C. E. and Weaver, W. (1949). The mathematical theory of communication (Urbana: University of Illinois Press). url: https://pure.mpg.de/rest/items/item_2383164/component/file_2383163/content.
- Shelton, H. M., Franzel, S., and Peters, M. (2005). Adoption of tropical legume technology around the world: Analysis of success. *Tropical Grasslands* 39(4), 198–209. url: http://www.tropicalgrasslands.asn.au/Tropical/%20Grasslands/%20Journal/%20archive/PDFs/Vol_39_2005/Vol_39_04_2005_pp198_209.pdf.
- Simpson, E. H. (1949). Measurement of diversity. *Nature* 163, 688–688. url: <https://www.nature.com/articles/163688a0>.
- Tilley, J. M. A. and Terry, R. A. (1963). A two-stage technique for the in vitro digestion of forage crops. *J. Br. Grassl. Soc* (18), 104–111. doi: <https://doi.org/10.1111/j.1365-2494.1963.tb00335.x>
- Van Soest, P. J., Robertson, J. B., and Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci* (74), 78551–78553. doi: [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2)
- Vogel, K. P., Pedersen, J. F., Masterson, S. D., and Toy, J. J. (1999). Evaluation of a filter bag system for NDF, ADF, and IVDMD forage analysis. *Crop Sci* (39), 276–279. url: <https://doi.org/10.2135/cropsci1999.0011183X003900010042x>.
- Wetzel, M. M. V. S., Silva, D. B., Salomão, A. N., Neto, L. G. P., and Faiad, M. G. R. (2012). Conservação de Germoplasma-Semente em Longo Prazo, ed. AM, C., CR, S., and JRB, S. (Brasília, DF: EMBRAPA), 160-184. url: <https://www.alice.cnptia.embrapa.br/handle/doc/998664>.



Phenotypic characterization of Gesha horses in southwestern Ethiopia

Amine Mustefa *, Aweke Engdawork, Seble Sinke and Abebe Hailu

Ethiopian Biodiversity Institute, Addis Ababa, Ethiopia

Abstract: Fifteen qualitative and 21 morphometric variables on a total of 394 adult horses (282 stallions and 112 mares) from three selected districts were recorded to characterize the horse populations in southwestern Ethiopia. General linear model, frequency, and multivariate analysis procedures of Statistical Analysis Software (SAS 9.0) were used to analyze the data. Sex and location significantly affected the studied traits. Stallions were larger than mares, and the Gesha horse population was the tallest, longest, and largest among the studied populations. The majority of the studied horses possess plain body colour patterns with red-coloured medium hair size. A higher frequency of white-coloured horses was observed with increasing age. Stepwise discriminant function analysis revealed that pelvic width, cannon bone length, and height at croup were the top three morphometric variables to discriminate the populations while head length, head neck circumference, chest width, cannon bone circumference, and croup length had the lowest discriminatory power. The results of discriminant function analysis showed advanced classification (76.7%) of the studied horses into their respective populations/locations. Finally, canonical discriminant function analysis categorized the horse populations into three distinct categories. The Gesha horse population was different from Masha and Telo horse populations while having a relatively higher relationship with the Masha horse population. However, the distances calculated in this study show only the relative size differences between each population. Such differences might not necessarily be due to breed (genetic) differences. Therefore, diversity studies through further genetic characterization are recommended to design conservation and breeding programmes.

Keywords: Ethiopia, Horse, Gesha, Phenotypic characterization

Citation: Mustefa, A., Engdawork, A., Sinke, S., Hailu, A. (2022). **Phenotypic characterization of Gesha horses in southwestern Ethiopia.** *Genetic Resources* 3 (5), 36–50. doi: [10.46265/genresj.KPIL8781](https://doi.org/10.46265/genresj.KPIL8781).

© Copyright 2022 the Authors.

This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

Horses are among the most important livestock species in the highlands of Ethiopia. In rural areas, horses are the main source of transportation, both for humans and agricultural goods. They are used in public events including social and cultural festivals, and are the most culturally respected and highly valued domestic animals in the country in general, and in southern and southwestern Ethiopia in particular (Kefena *et al*, 2012). The highlands of Keffa and Sheka zones in southwest Ethiopia are also among the most benefitted areas from the indigenous horses (Kefena *et al*, 2012). In these areas, horses were also used for traditional racing shows.

Ethiopia is reported to possess 2.1 million horses (Central Statistical Agency, 2020). However, in terms of standard characterization and documentation, the equine sector has received little attention. Until now, only one country-wide general study by Kefena *et al* (2012) was performed to phenotypically characterize the country's horse breeds, their geographical distribution and production environments. Accordingly, eight breeds (Abyssinian, Bale, Boran, Horro, Kafa, Kundo feral horse, Ogaden/Wilwal and Selale horse) were officially reported to exist in the country (Kefena *et al*, 2012; EBI, 2016).

However, due to different reasons, the study by Kefena *et al* (2012) did not cover or characterize three horse breeds (Boran, Kundo feral horse and Ogaden/Wilwal horses) out of the total eight breeds. Additionally, the lack of qualitative morphological data in the study, and the small sample size taken (95–106

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

horses per breed) can be noted as limitations of the study. Similarly, the selected sampling sites were too narrow to represent the horse populations of the area. For example, the horse populations of southwestern Ethiopia were represented by a sample from a single site (Masha district). A preliminary study by a team of livestock experts from Keffa zone hinted at the presence of an unstudied unique horse population in Gesha district.

According to the results of this preliminary study, Gesha horses are said to be typical riding horses of the Keffa zone highlands. However, in the country-wide study by Kefena *et al* (2012), this population was represented by horses from the neighbouring Masha district. Therefore, further characterization studies were required to better understand the horse populations and quantify the level of relationships among them, thus providing a clear country-wide picture. Hence, the current study was designed to characterize the horse populations in southwestern Ethiopia using both quantitative morphometric measurements and qualitative morphological characteristics.

Materials and methods

Locations

This study was conducted in Keffa and Sheka zones of the Southern Nations Nationalities and Peoples Regional State (SNNPR), Ethiopia. Three locations were selected for the current study (Table 1, Figure 1). Gesha and Masha districts were sampled purposively: Gesha district (one of the ten districts in Keffa zone) is the location of the horses which were supposed to be unique and unaddressed before, while Masha district (one of the three districts in Sheka zone) is where the samples were taken for the previous country-wide study by Kefena *et al* (2012). Telo district was sampled randomly from Keffa zone to study the relationship of its horses with Gesha horses.

The sampling frame was defined after collecting available background information (origin, distribution, population size, and unique features) of the unstudied horse population through focus group discussions with livestock keepers and experts. Additionally, information regarding the sampling sites of the country-wide study was also taken from the reports of Kefena *et al* (2012).

Data collection

Quantitative and qualitative data were recorded from a total of 394 adult horses (282 stallions and 112 mares) based on the data collection procedures outlined in FAO (2012) and the previous country-wide study by Kefena *et al* (2012). Studied horses were carefully handled by their owners and trained personnel. Data were collected when the animals were calm and standing in an upright position on flat ground and early in the morning of the day before feeding and watering. To minimize measurement error, data were not taken from aggressive horses that did not stand properly. Similarly, to minimize subjectivity error, measurements and data recording

were performed by the same researchers throughout the study. A centimetre-unit textile measuring tape was used for the morphometric measurements.

Twenty-one quantitative morphometric measurements (Table 2) and 15 qualitative characteristics (hair size, body colour pattern, colour of the body, head, muzzle, tail and hoof, presence/absence of stripe at dorsal body, shoulder and leg, profile of the face, back and croup, length of the tail and mane) were collected.

The following body measure indices were calculated from morphometric measurements (adapted from Bodó and Hecker (1992); Cabral *et al* (2004); Druml *et al* (2008); Bene *et al* (2013)).

- Body index = (Body length/Thorax girth) x 100
- Quadratic index = (Height at withers/Body length) x 100
- Caliber index = (Thorax girth/Height at withers) x (Cannon circumference/Height at withers) x 1000
- Overbuilt index = (Height at croup/Height at withers) x 100
- Chest index = (Chest width/Thorax girth) x 100
- Conformation index = (Thorax girth²/Height at withers)/100

Data analysis

Data entry and management were performed using Microsoft Excel® worksheet. Analysis of the quantitative traits was performed separately for stallions, mares and sex-aggregated by fitting location and age as fixed variables. UNIVARIATE procedure of Statistical Analysis Software (SAS) 9.0 was used to detect outliers and test the normality of morphometric data (SAS Institute, 2002). Data on qualitative traits were subjected to chi-square (χ^2) tests of the frequency (FREQ) procedure of SAS 9.0 software. Quantitative morphometric and body measure indices data were analyzed using the general linear model (GLM) procedure of SAS 9.0 software, with adjusted Tukey-Kramer test to separate the least square means (LSM). Data analysis was performed using the following model: $Y_{ijk} = \mu + S_i + L_j + A_k + e_{ijk}$ where Y_{ijk} is an observation, μ is the overall mean, S_i is the fixed effect of i^{th} sex (i = stallion, mare), L_j is the fixed effect of j^{th} location (j = Telo, Gesha, Masha), A_k is the fixed effect of k^{th} age (k = 4–11), and e_{ijk} is the random error attributed to the n^{th} observation. The sex effect was removed from the class variables when the analysis was done separately for each sex.

Morphometric traits that better discriminate the horse populations from different locations were identified using the forward selection method of the stepwise discriminant function analysis (STEPDISC) procedure of SAS 9.0. The discriminant function analysis (DISCRIM) procedure of SAS 9.0. was also used to assign observations to locations and evaluate probabilities of misclassifications. Linear combination of morphometric variables that provide maximal separations between locations was performed using the canonical discriminant function analysis (CANDISC) procedure of SAS 9.0. The scored canonical variables were used to plot

Table 1. Climatic and agroecological features of the studied areas. Data from Bezabih (2012), Assefa et al (2013), Gebrmichael (2019).

Climate factors	Telo	Gesha	Masha
Altitude (m)	2,436–2,451	1,501–3,000	1,700–3,000
Temperature (°C)	17–25	15.1–20	16.7
Rainfall (mm)	1,278	2,001–2,200	2,192
Agroecology	Highland	Midland and highland	Midland and highland

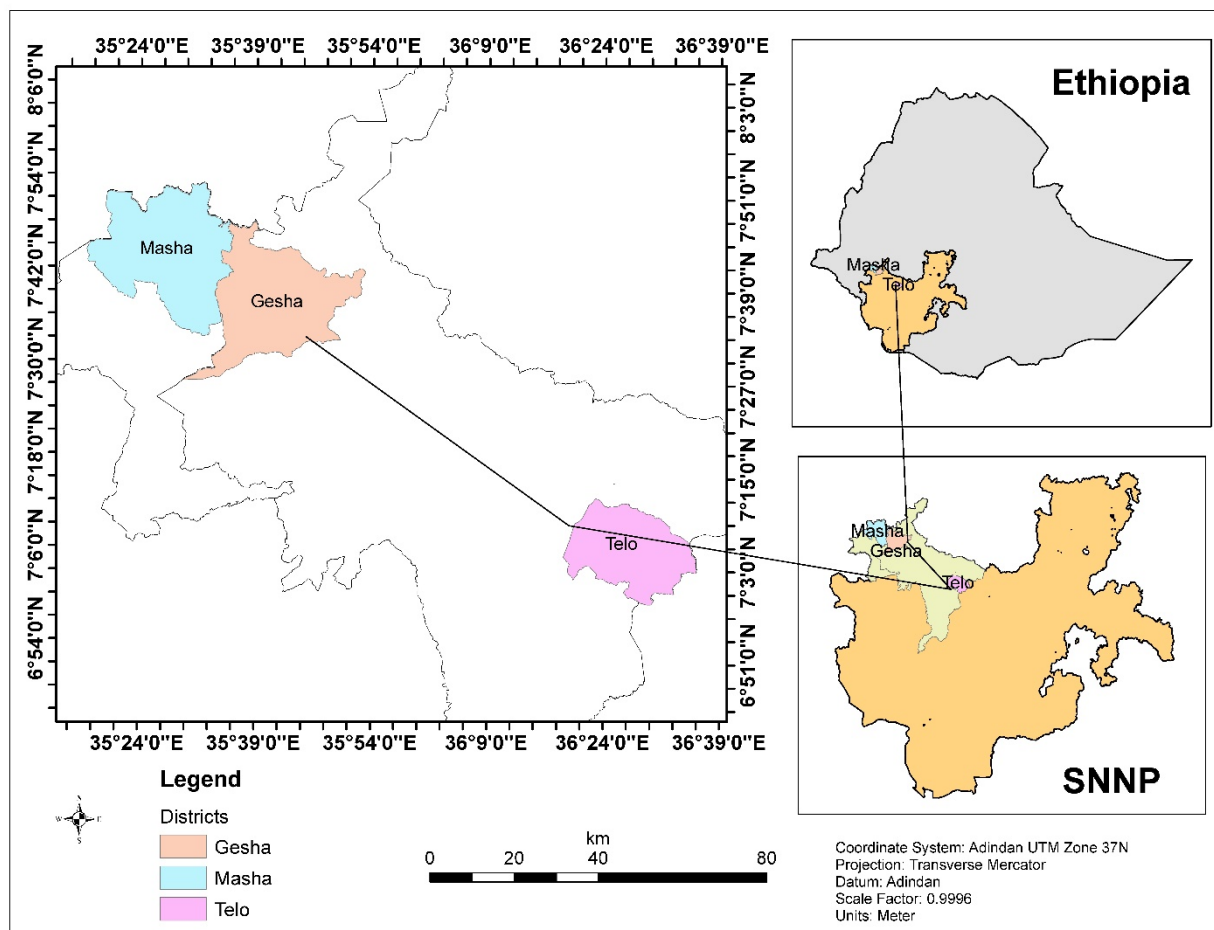


Figure 1. Map of the sampled locations and districts

pairs of canonical variables to get visual interpretation of location differences. Pairwise squared Mahalanobis distances between locations were computed as: $D^2(i|j) = (x_i - x_j)' cov^{-1} (x_i - x_j)$. Where $D^2(i|j)$ is the distances between locations 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} populations.

Results

Morphometric measurements and body measure indices

The effect of sex on the studied morphometric variables is presented in Table 3. Most measurements were higher for stallions than mares while ear length and barrel

length measurements of the mares were higher than the stallions. On the other hand, body length and back length measurements were not significantly affected by sex.

To have a clear picture of the differences among locations, the analysis was performed separately for both sexes. The effect of location on the morphometric measurements of the stallions is presented in Table 4. All stallions' measurements were affected significantly by their location. Gesha stallions had significantly the highest values for most of the measurements except for cannon bone length where Telo stallions had higher values. Masha stallions had relatively higher measurement values than their counterparts from Telo district, and these populations shared more similarities. On the other hand, chest width, shoulder depth, body

Table 2. Description of the collected quantitative measurements. Adapted from [FAO \(2012\)](#); [Kefena et al \(2012\)](#).

No.	Morphometric measurements	Explanation of the measurements
1	Head length	Distance from the nape to the alveolar edge of the incisors I of the upper jaw
2	Head width	Distance between the upper side of the eyes measured perpendicularly to the head length
3	Ear length	Distance from the tip of the ear to the connection point with the head
4	Head neck circumference	Circumference of the neck at the connection point to the head
5	Neck length	Distance from the highest point of the withers to the nape with the neck in a relaxed position
6	Neck body circumference	Circumference of the neck at the connection point with the body
7	Chest width	Distance between two outer points of the humeral bones from the front
8	Shoulder depth	Distance from the withers to the shoulder joint
9	Thorax depth	Distance from the withers to the sternum
10	Thorax width	Distance between two hypothetical vertical parallel lines drawn at the thorax sides and along the withers' height line
11	Thorax girth	Measured in the place of the saddle girth
12	Cannon bone length	Distance from the lateral tuberculum of the os metacarpale IV to the fetlock joint
13	Cannon bone circumference	Smallest circumference of the forelimb's cannon bone
14	Height at wither	Distance from the highest point of the processus spinalis of the vertebra thoracic to the floor
15	Height at back	Distance from the deepest point of the back to the floor
16	Height at croup	Distance from the croup (rump) to the floor
17	Body length	Distance from the most cranial point of the shoulder joint to the most caudal point of the pin bone (scapulo-ischial length)
18	Back length	Distance from the caudal point of the shoulder joint perpendicular to the wither to the most cranial point of the hip joint measured in the saddle place
19	Pelvic width	Distance between the right and left coxal tubers of the ilium
20	Croup length	Distance between the sacral tuber (the highest point of croup) and ischiatic tuber (most posterior point of ischium or point of buttock or seat bone)
21	Barrel length	Distance from the most caudal point of the scapula to the most cranial and dorsal point of the point of the hip

length and back length measurements of Telo stallions were higher than Masha stallions.

The effect of location on the morphometric measurements of the mares is presented in [Table 5](#). Most of the mares' measurements were affected significantly by their location except ear length, neck length, chest width and barrel length. Gesha mares were the biggest and heaviest among the studied populations: their circumferences of head-neck, neck-body and thorax, and heights at withers, back and croup, and pelvic width were significantly larger than Telo or Masha.

The effect of location on the morphometric measurements of the studied horse populations (sex-aggregated) is presented in [Table 6](#). All the morphometric measurements of the studied horse populations were affected significantly by their location. Significantly, the Gesha horse population had the highest values for most of the measurements except for cannon bone length, which was higher in Telo horses.

Pearson correlation coefficients of the morphometric measurements of the horses (both sexes) from different locations are presented in [Table 7](#). The majority of the traits were positively correlated. Higher positive correlation was observed between height at withers and height at back while lower positive correlation was observed

between ear length and head neck circumference. Negative correlation was observed between thorax width and cannon bone length.

The effect of location on body measure indices of the studied horse populations (separately for each sex) is presented in [Table 8](#). All the body measure indices of the studied horse populations were significantly affected by sex. Similarly, most of the body measure indices were significantly affected by their location.

Multivariate analysis

Stepwise discriminant function analysis revealed the order of importance of the studied morphometric variables in discriminating the horse populations ([Table 9](#)). The results were also confirmed by Wilk's lambda test ([Table 9](#)) where all the selected variables had a highly significant ($P < 0.0001$) contribution in discriminating the horse populations. Pelvic width, cannon bone length and height at croup were the first three important traits used in discriminating the studied horse populations. However, some morphometric variables like head length, head neck circumference, chest width, cannon bone circumference and croup length had the lowest dis-

Table 3. Least-square means $\pm$ standard errors of quantitative body measurements (cm) of the horse populations by sex.

Traits	Stallions	Mares	p-value
N	282	112	
Head length	53.2 $\pm$ 0.16	52.1 $\pm$ 0.23	< 0.0001
Head width	21.5 $\pm$ 0.06	21.2 $\pm$ 0.09	0.0015
Ear length	15.0 $\pm$ 0.09	15.5 $\pm$ 0.13	0.0033
Head neck circumference	58.9 $\pm$ 0.24	54.7 $\pm$ 0.36	< 0.0001
Neck length	59.4 $\pm$ 0.27	57.3 $\pm$ 0.40	< 0.0001
Neck body circumference	91.4 $\pm$ 0.37	84.3 $\pm$ 0.55	< 0.0001
Chest width	25.9 $\pm$ 0.13	24.3 $\pm$ 0.20	< 0.0001
Shoulder depth	53.4 $\pm$ 0.18	51.1 $\pm$ 0.27	< 0.0001
Thorax depth	61.8 $\pm$ 0.23	59.8 $\pm$ 0.33	< 0.0001
Thorax width	34.2 $\pm$ 0.16	32.9 $\pm$ 0.24	< 0.0001
Thorax girth	143.0 $\pm$ 0.44	138.6 $\pm$ 0.65	< 0.0001
Cannon bone length	24.0 $\pm$ 0.09	23.6 $\pm$ 0.14	0.0066
Cannon bone circumference	16.4 $\pm$ 0.06	15.6 $\pm$ 0.09	< 0.0001
Height at withers	131.8 $\pm$ 0.29	127.8 $\pm$ 0.43	< 0.0001
Height at back	129.0 $\pm$ 0.28	125.7 $\pm$ 0.41	< 0.0001
Height at croup	132.1 $\pm$ 0.28	129.1 $\pm$ 0.42	< 0.0001
Body length	125.0 $\pm$ 0.38	124.1 $\pm$ 0.56	0.1796
Back length	70.0 $\pm$ 0.26	70.2 $\pm$ 0.38	0.6114
Pelvic width	40.4 $\pm$ 0.16	39.5 $\pm$ 0.24	0.0016
Croup length	39.7 $\pm$ 0.18	38.6 $\pm$ 0.27	0.0020
Barrel length	67.0 $\pm$ 0.29	69.4 $\pm$ 0.43	< 0.0001

Table 4. Means and pairwise comparisons of morphometric measurements of the stallions from different locations. Means within a row bearing different superscripts are significantly different; ^a indicates the largest value.

Traits	Least Square Means (LSM $\pm$ SE)			Mean $\pm$ SE	CV	p-value
	Telo	Gesha	Masha			
N	94	136	52			
Head length	52.4 $\pm$ 0.27 ^b	54.2 $\pm$ 0.23 ^a	53.1 $\pm$ 0.36 ^b	53.2 $\pm$ 0.16	4.5	< 0.0001
Head width	21.0 $\pm$ 0.11 ^b	21.9 $\pm$ 0.09 ^a	21.6 $\pm$ 0.15 ^a	21.5 $\pm$ 0.07	4.6	< 0.0001
Ear length	14.5 $\pm$ 0.15 ^b	15.1 $\pm$ 0.13 ^a	15.4 $\pm$ 0.20 ^a	14.9 $\pm$ 0.08	8.9	0.0002
Head neck circumference	58.1 $\pm$ 0.43 ^b	60.8 $\pm$ 0.36 ^a	57.9 $\pm$ 0.57 ^b	59.3 $\pm$ 0.24	6.4	< 0.0001
Neck length	59.3 $\pm$ 0.48 ^b	60.6 $\pm$ 0.40 ^a	58.4 $\pm$ 0.63 ^b	59.4 $\pm$ 0.27	7.1	0.0049
Neck body circumference	88.8 $\pm$ 0.63 ^b	95.9 $\pm$ 0.53 ^a	89.9 $\pm$ 0.83 ^b	92.2 $\pm$ 0.41	6.1	< 0.0001
Chest width	26.0 $\pm$ 0.25 ^{ab}	26.7 $\pm$ 0.21 ^a	25.2 $\pm$ 0.33 ^b	26.0 $\pm$ 0.14	8.5	0.0008
Shoulder depth	53.5 $\pm$ 0.32 ^b	54.6 $\pm$ 0.27 ^a	52.1 $\pm$ 0.42 ^c	53.6 $\pm$ 0.18	5.3	< 0.0001
Thorax depth	59.6 $\pm$ 0.40 ^c	63.7 $\pm$ 0.33 ^a	61.9 $\pm$ 0.52 ^b	61.9 $\pm$ 0.25	5.7	< 0.0001
Thorax width	32.8 $\pm$ 0.30 ^c	35.5 $\pm$ 0.25 ^a	34.2 $\pm$ 0.39 ^b	34.1 $\pm$ 0.18	7.7	< 0.0001
Thorax girth	141.5 $\pm$ 0.78 ^b	149.0 $\pm$ 0.65 ^a	138.8 $\pm$ 1.02 ^b	143.6 $\pm$ 0.53	4.8	< 0.0001
Cannon bone length	24.8 $\pm$ 0.16 ^a	23.5 $\pm$ 0.14 ^b	23.6 $\pm$ 0.21 ^b	24.0 $\pm$ 0.09	6.0	< 0.0001
Cannon bone circumference	16.34 $\pm$ 0.11 ^b	16.9 $\pm$ 0.10 ^a	16.1 $\pm$ 0.15 ^b	16.5 $\pm$ 0.07	6.1	< 0.0001
Height at withers	130.7 $\pm$ 0.51 ^b	135.2 $\pm$ 0.43 ^a	129.6 $\pm$ 0.67 ^b	132.2 $\pm$ 0.31	3.4	< 0.0001
Height at back	127.8 $\pm$ 0.48 ^b	132.4 $\pm$ 0.41 ^a	127.0 $\pm$ 0.63 ^b	129.5 $\pm$ 0.30	3.3	< 0.0001
Height at croup	131.3 $\pm$ 0.50 ^b	135.8 $\pm$ 0.42 ^a	129.6 $\pm$ 0.7 ^b	132.8 $\pm$ 0.31	3.3	< 0.0001
Body length	125.3 $\pm$ 0.67 ^b	127.5 $\pm$ 0.56 ^a	122.4 $\pm$ 0.88 ^c	125.3 $\pm$ 0.38	4.7	< 0.0001
Back length	70.7 $\pm$ 0.44 ^a	71.1 $\pm$ 0.37 ^a	68.2 $\pm$ 0.58 ^b	70.1 $\pm$ 0.25	5.6	0.0003
Pelvic width	38.7 $\pm$ 0.29 ^c	42.3 $\pm$ 0.24 ^a	40.5 $\pm$ 0.38 ^b	40.5 $\pm$ 0.19	6.3	< 0.0001
Croup length	38.7 $\pm$ 0.33 ^b	41.1 $\pm$ 0.28 ^a	39.3 $\pm$ 0.43 ^b	39.7 $\pm$ 0.18	7.3	< 0.0001
Barrel length	66.1 $\pm$ 0.51 ^b	67.7 $\pm$ 0.43 ^a	67.1 $\pm$ 0.66 ^{ab}	66.8 $\pm$ 0.28	6.7	0.0287

Table 5. Means and pairwise comparisons of morphometric measurements of the mares from different locations. Means within a row bearing different superscripts are significantly different; ^a indicates the largest value.

Traits	Least Square Means (LSM ± SE)			Mean ± SE	CV	p-value
	Telo	Gesha	Masha			
N	29	47	36			
Head length	51.9 ± 0.49 ^{ab}	53.0 ± 0.38 ^a	51.3 ± 0.45 ^b	52.1 ± 0.25	4.9	0.0128
Head width	20.6 ± 0.18 ^b	21.5 ± 0.14 ^a	21.5 ± 0.17 ^a	21.2 ± 0.10	4.4	0.0001
Ear length	15.2 ± 0.27	15.4 ± 0.21	15.9 ± 0.25	15.5 ± 0.13	9.1	0.1536
Head neck circumference	53.2 ± 0.70 ^b	56.7 ± 0.53 ^a	54.3 ± 0.64 ^b	54.9 ± 0.37	6.6	0.0002
Neck length	56.7 ± 0.75	58.4 ± 0.57	56.5 ± 0.69	57.2 ± 0.38	6.8	0.0597
Neck body circumference	81.3 ± 1.20 ^b	89.4 ± 0.91 ^a	81.7 ± 1.10 ^b	84.7 ± 0.71	7.3	< 0.0001
Chest width	23.9 ± 0.32	24.8 ± 0.24	24.0 ± 0.29	24.3 ± 0.16	6.8	0.0535
Shoulder depth	51.0 ± 0.54 ^{ab}	52.2 ± 0.41 ^a	49.9 ± 0.49 ^b	51.0 ± 0.30	5.4	0.0015
Thorax depth	58.7 ± 0.63 ^b	61.1 ± 0.48 ^a	59.7 ± 0.58 ^{ab}	59.9 ± 0.34	5.4	0.0069
Thorax width	31.4 ± 0.44 ^b	34.1 ± 0.34 ^a	33.2 ± 0.41 ^a	32.9 ± 0.27	7.0	< 0.0001
Thorax girth	135.9 ± 1.26 ^b	144.4 ± 0.96 ^a	134.6 ± 1.16 ^b	138.6 ± 0.81	4.7	< 0.0001
Cannon bone length	24.1 ± 0.27 ^a	22.9 ± 0.21 ^b	23.6 ± 0.25 ^{ab}	23.5 ± 0.14	6.0	0.0014
Cannon bone circumference	15.6 ± 0.15 ^{ab}	15.9 ± 0.11 ^a	15.4 ± 0.13 ^b	15.6 ± 0.08	4.9	0.0067
Height at withers	127.3 ± 0.81 ^b	130.3 ± 0.62 ^a	125.4 ± 0.75 ^b	127.9 ± 0.45	3.3	< 0.0001
Height at back	124.8 ± 0.84 ^b	128.4 ± 0.64 ^a	123.4 ± 0.77 ^b	125.8 ± 0.46	3.5	< 0.0001
Height at croup	128.3 ± 0.81 ^b	132.1 ± 0.61 ^a	126.3 ± 0.74 ^b	129.3 ± 0.47	3.2	< 0.0001
Body length	124.5 ± 1.13 ^{ab}	126.6 ± 0.86 ^a	121.0 ± 1.04 ^b	124.0 ± 0.65	4.7	0.0004
Back length	70.6 ± 0.80 ^{ab}	71.4 ± 0.61 ^a	68.1 ± 0.74 ^b	70.0 ± 0.44	6.0	0.0033
Pelvic width	38.6 ± 0.43 ^b	41.4 ± 0.33 ^a	38.4 ± 0.40 ^b	39.6 ± 0.27	5.7	< 0.0001
Croup length	38.8 ± 0.52 ^{ab}	39.6 ± 0.39 ^a	37.7 ± 0.47 ^b	38.7 ± 0.27	6.9	0.0120
Barrel length	68.8 ± 0.89	70.2 ± 0.68	69.4 ± 0.82	69.3 ± 0.47	6.7	0.4347

Table 6. Means and pairwise comparisons of morphometric measurements of the horses (both sexes) from different locations. Means within a row bearing different superscripts are significantly different; ^a indicates the largest value.

Traits	Least Square Means (LSM ± SE)			Mean ± SE	CV	p-value
	Telo	Gesha	Masha			
N	123	183	88			
Head length	52.0 ± 0.24 ^b	53.6 ± 0.20 ^a	52.2 ± 0.27 ^b	52.9 ± 0.14	4.6	< 0.0001
Head width	20.8 ± 0.10 ^b	21.7 ± 0.08 ^a	21.6 ± 0.11 ^a	21.4 ± 0.06	4.6	< 0.0001
Ear length	14.8 ± 0.14 ^b	15.3 ± 0.11 ^a	15.7 ± 0.15 ^a	15.1 ± 0.07	8.9	< 0.0001
Head neck circumference	55.8 ± 0.38 ^b	58.7 ± 0.31 ^a	56.0 ± 0.41 ^b	58.1 ± 0.23	6.5	< 0.0001
Neck length	58.1 ± 0.42 ^b	59.5 ± 0.34 ^a	57.4 ± 0.46 ^b	58.8 ± 0.23	7.0	0.0004
Neck body circumference	85.1 ± 0.58 ^b	92.5 ± 0.48 ^a	86.0 ± 0.64 ^b	90.1 ± 0.39	6.4	< 0.0001
Chest width	25.0 ± 0.21 ^b	25.7 ± 0.17 ^a	24.6 ± 0.23 ^b	25.5 ± 0.12	8.1	< 0.0001
Shoulder depth	52.3 ± 0.28 ^b	53.4 ± 0.23 ^a	51.0 ± 0.31 ^c	52.8 ± 0.17	5.3	< 0.0001
Thorax depth	58.9 ± 0.35 ^c	62.6 ± 0.29 ^a	61.0 ± 0.39 ^b	61.3 ± 0.21	5.7	< 0.0001
Thorax width	32.1 ± 0.25 ^c	34.8 ± 0.21 ^a	33.7 ± 0.28 ^b	33.8 ± 0.15	7.5	< 0.0001
Thorax girth	138.9 ± 0.68 ^b	146.6 ± 0.56 ^a	136.9 ± 0.75 ^b	142.2 ± 0.46	4.8	< 0.0001
Cannon bone length	24.5 ± 0.14 ^a	23.2 ± 0.12 ^b	23.6 ± 0.16 ^b	23.9 ± 0.08	6.0	< 0.0001
Cannon bone circumference	15.9 ± 0.09 ^b	16.4 ± 0.08 ^a	15.7 ± 0.10 ^b	16.2 ± 0.06	5.8	< 0.0001
Height at withers	128.8 ± 0.45 ^b	132.8 ± 0.37 ^a	127.7 ± 0.50 ^b	131.0 ± 0.28	3.4	< 0.0001
Height at back	126.1 ± 0.43 ^b	130.4 ± 0.36 ^a	125.5 ± 0.48 ^b	128.4 ± 0.26	3.4	< 0.0001
Height at croup	129.7 ± 0.44 ^b	134.0 ± 0.36 ^a	128.2 ± 0.48 ^b	131.8 ± 0.27	3.3	< 0.0001
Body length	124.8 ± 0.59 ^b	127.0 ± 0.49 ^a	121.7 ± 0.65 ^c	124.9 ± 0.33	4.7	< 0.0001
Back length	70.8 ± 0.40 ^a	71.2 ± 0.33 ^a	68.2 ± 0.44 ^b	70.1 ± 0.22	5.7	< 0.0001
Pelvic width	38.4 ± 0.25 ^c	41.8 ± 0.21 ^a	39.6 ± 0.28 ^b	40.3 ± 0.16	6.3	< 0.0001
Croup length	38.4 ± 0.29 ^b	40.4 ± 0.23 ^a	38.6 ± 0.32 ^b	39.4 ± 0.16	7.2	< 0.0001
Barrel length	67.4 ± 0.45 ^b	69.0 ± 0.37 ^a	68.3 ± 0.50 ^b	67.5 ± 0.25	6.6	0.0113

Table 7. Pearson correlation coefficients between each morphometric measurement (above diagonal) and level of significance (below diagonal) of the horses (both sexes) from the three locations. HL, Head length; HW, Head width; EL, Ear length; HNC, Head neck circumference; NL, Neck length; NBC, Neck body circumference; CW, Chest width; SD, Shoulder depth; TD, Thorax depth; TW, Thorax width; TG, Thorax girth; CBL, Cannon bone length; CBC, Cannon bone circumference; HAW, Height at withers; HAB, Height at back; HAC, Height at croup; BOL, Body length; BAL, Back length; PW, Pelvic width; CL, Croup length; BRL, Barrel length. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.0001$; NS, Not Significant.

Traits	HL	HW	EL	HNC	NL	NBC	CW	SD	TD	TW	TG	CBL	CBC	HAW	HAB	HAC	BOL	BAL	PW	CL	BRL
HL		0.48	0.16	0.35	0.22	0.48	0.41	0.49	0.51	0.38	0.55	0.08	0.41	0.59	0.56	0.56	0.39	0.36	0.42	0.28	0.29
HW	***		0.24	0.37	0.30	0.44	0.39	0.41	0.46	0.44	0.48	0.06	0.36	0.42	0.42	0.41	0.36	0.30	0.42	0.35	0.31
EL	**	***		0.001	0.14	0.07	0.14	0.08	0.17	0.20	0.11	0.03	0.10	0.11	0.11	0.11	0.11	0.16	0.19	0.20	0.23
HNC	***	***	NS		0.34	0.74	0.49	0.57	0.46	0.48	0.67	0.12	0.53	0.57	0.52	0.52	0.43	0.19	0.49	0.47	0.11
NL	***	***	**	***		0.49	0.35	0.42	0.39	0.39	0.53	0.13	0.44	0.53	0.53	0.54	0.47	0.32	0.38	0.40	0.23
NBC	***	***	NS	***	***		0.55	0.66	0.62	0.56	0.78	0.06	0.61	0.70	0.67	0.67	0.49	0.31	0.60	0.52	0.16
CW	***	***	**	***	***	***		0.56	0.45	0.41	0.59	0.23	0.47	0.47	0.41	0.43	0.41	0.40	0.45	0.40	0.25
SD	***	***	NS	***	***	***	***		0.57	0.47	0.73	0.20	0.55	0.69	0.65	0.66	0.56	0.39	0.49	0.51	0.29
TD	***	***	**	***	***	***	***	***		0.56	0.67	0.06	0.52	0.62	0.61	0.59	0.42	0.32	0.56	0.44	0.30
TW	***	***	***	***	***	***	***	***	***		0.66	-0.04	0.49	0.54	0.54	0.52	0.47	0.30	0.57	0.48	0.33
TG	***	***	*	***	***	***	***	***	***	***		0.09	0.66	0.78	0.76	0.76	0.67	0.47	0.72	0.60	0.36
CBL	NS	NS	NS	*	*	NS	***	***	NS	NS	NS		0.26	0.16	0.14	0.15	0.15	0.22	0.002	0.09	0.05
CBC	***	***	NS	***	***	***	***	***	***	***	***	***		0.63	0.60	0.61	0.51	0.39	0.51	0.44	0.20
HAW	***	***	*	***	***	***	***	***	***	***	***	**	***		0.96	0.94	0.60	0.45	0.60	0.55	0.28
HAB	***	***	*	***	***	***	***	***	***	***	***	**	***	***		0.93	0.57	0.43	0.57	0.54	0.28
HAC	***	***	*	***	***	***	***	***	***	***	***	**	***	***	***		0.63	0.47	0.59	0.56	0.31
BOL	***	***	*	***	***	***	***	***	***	***	***	**	***	***	***	***		0.51	0.54	0.50	0.58
BAL	***	***	**	**	***	***	***	***	***	***	***	**	***	***	***	***	***		0.41	0.29	0.37
PW	***	***	**	***	***	***	***	***	***	***	***	NS	***	***	***	***	***	***		0.59	0.35
CL	***	***	***	***	***	***	***	***	***	***	***	NS	***	***	***	***	***	***	***		0.26
BRL	***	***	***	*	***	**	***	***	***	***	***	NS	***	***	***	***	***	***	***	***	

Table 8. Body measure indices of the studied horse populations

Traits	Least Square Means (LSM $\pm$ SE)			Mean $\pm$ SE	p-value
	Telo	Gesha	Masha		
Stallions					
Body index	88.71 $\pm$ 0.40 ^a	85.60 $\pm$ 0.34 ^b	88.33 $\pm$ 0.53 ^a	87.36 $\pm$ 0.24	< 0.0001
Quadratic index	104.5 $\pm$ 0.47 ^b	106.2 $\pm$ 0.40 ^a	106.0 $\pm$ 0.62 ^{ab}	105.7 $\pm$ 0.25	0.0095
Caliber index	135.4 $\pm$ 1.16 ^{ab}	138.0 $\pm$ 0.98 ^a	132.8 $\pm$ 1.53 ^b	135.5 $\pm$ 0.65	0.0119
Overbuilt index	100.4 $\pm$ 0.16	100.5 $\pm$ 0.14	100.0 $\pm$ 0.21	100.4 $\pm$ 0.09	0.2523
Chest index	18.40 $\pm$ 0.15 ^a	17.89 $\pm$ 0.13 ^b	18.17 $\pm$ 0.20 ^{ab}	18.1 $\pm$ 0.08	0.0178
Conformation index	1.53 $\pm$ 0.014 ^b	1.65 $\pm$ 0.011 ^a	1.49 $\pm$ 0.018 ^b	1.56 $\pm$ 0.009	< 0.0001
Mares					
Body index	91.67 $\pm$ 0.75 ^a	87.75 $\pm$ 0.58 ^b	90.00 $\pm$ 0.69 ^a	89.57 $\pm$ 0.40	0.0002
Quadratic index	102.3 $\pm$ 0.79	103.2 $\pm$ 0.60	103.9 $\pm$ 0.72	103.4 $\pm$ 0.42	0.3730
Caliber index	130.7 $\pm$ 1.57	135.3 $\pm$ 1.20	131.4 $\pm$ 1.44	132.1 $\pm$ 0.89	0.0580
Overbuilt index	100.8 $\pm$ 0.25	101.4 $\pm$ 0.19	100.7 $\pm$ 0.23	101.0 $\pm$ 0.13	0.0617
Chest index	17.61 $\pm$ 0.21 ^{ab}	17.23 $\pm$ 0.16 ^b	17.88 $\pm$ 0.19 ^a	17.53 $\pm$ 0.10	0.0379
Conformation index	1.45 $\pm$ 0.02 ^b	1.60 $\pm$ 0.02 ^a	1.45 $\pm$ 0.02 ^b	1.50 $\pm$ 0.01	< 0.0001
Both sexes					
Body index	90.01 $\pm$ 0.37 ^a	86.71 $\pm$ 0.30 ^b	89.07 $\pm$ 0.41 ^a	87.99 $\pm$ 0.21	< 0.0001
Quadratic index	103.3 $\pm$ 0.42 ^b	104.8 $\pm$ 0.34 ^a	105.05 $\pm$ 0.46 ^a	105.1 $\pm$ 0.22	0.0036
Caliber index	133.5 $\pm$ 0.97 ^b	136.6 $\pm$ 0.80 ^a	131.9 $\pm$ 1.07 ^b	134.5 $\pm$ 0.53	0.0005
Overbuilt index	100.7 $\pm$ 0.14	100.9 $\pm$ 0.12	100.4 $\pm$ 0.16	100.6 $\pm$ 0.07	0.0579
Chest index	18.02 $\pm$ 0.13 ^a	17.55 $\pm$ 0.10 ^b	17.99 $\pm$ 0.14 ^a	17.95 $\pm$ 0.07	0.0019
Conformation index	1.50 $\pm$ 0.01 ^b	1.62 $\pm$ 0.01 ^a	1.47 $\pm$ 0.01 ^b	1.54 $\pm$ 0.01	< 0.0001

criminatory power and were not used in discriminating the horse populations.

The values and significant levels of different statistical tests used in the discriminant function analysis are shown in Table 10. All the statistical tests were significant showing the appropriateness of the model used in discriminating the horse populations.

Outputs of the canonical discrimination analysis including eigenvalues and class means under the first two canonical structures are presented in Table 11. Similarly, Table 11 also presents raw canonical coefficients used in constructing the two canonical variables (Can 1 and Can 2). Accordingly, the first canonical structure (Can 1) explained the majority (65.7%) of the total variability among the three horse populations. It also produced a greater eigenvalue and multiple correlation (0.70) between the classes (locations) and the morphometric measurements than the second canonical structure (Can 2). These results show the higher power of Can 1 compared with Can 2 in separating the horse populations from the studied locations. However, Can 2 also separated one-third of the population, which Can 1 is unable to separate. Accordingly, Can 1 separated Telo horses from the others while Can 2 separated Masha horses from the others.

Discriminant function analysis classified each individual observation into a known population/location (Table 12). Accordingly, an average of 76.7% of the sampled animals were classified into their respective population/location. The highest classification of individual horses into their respective locations was observed in the Telo horse population (79.7%) with a small error rate (20.3%). On the other hand, a high error rate (26.1%) was detected in the Masha horse population. The priors (33.3%) show the chance of every individual observation to be classified into the given three populations/locations.

Pairwise squared Mahalanobis distances between locations are shown in Table 13. All distances were significant. Gesha and Masha horse populations are closely related, while their distance from the Telo horse population is large.

A plot of the first two canonical structures discriminating the studied horse populations is presented in Figure 2. Accordingly, Can 1 separates the Telo horse population from the others, while Can 2 discriminates the Masha horse population from the others. Overall, the analysis categorized the horse populations into three distinct categories. Therefore, the Gesha horse population is different from the Masha and Telo horse populations. Furthermore, the Gesha horse population has more relationship with the Masha than the Telo horse population.

Qualitative characteristics

Chi-square and Cramér's V statistical values and level of significance for the effect of the class variables on the qualitative characteristics of the studied horse populations are presented in Table 14. All the traits

were significantly affected by the location of the horse populations except body colour pattern and shoulder stripe. On the other hand, only five traits were significantly affected by the horses' sex and age. Face and back profile of the studied horse populations were found to be highly associated with location while the level of relationship of shoulder stripe with location was insignificant. A higher level of relationship between the horses' sex and age with their head colour was also observed.

The majority of the studied horse populations possess a plain body colour pattern with red, medium hair size, and long tail and mane with a mainly black muzzle, tail and hoof (Tables 15 and 16, Figure 3). All horses had sloppy croup with the absence of leg stripe. Short hair size, convex face and straight back profiles were observed more frequently on stallions than mares. The majority of the Gesha horses had red body and head (Figure 3, C and D) while white-striped red head was also frequently observed. White body and head colour were observed more frequently on Telo horses. Around half of the horse population from Masha district had black and white hoof, which was rarely observed in the other horse populations.

The effect of age on the colour-related qualitative characteristics of the studied horse populations is presented in Figure 4. Little effect of age on the colour-related qualitative characteristics was observed. As the age of the studied horses increased, the proportion of horses with white body colour showed a significant increase ($p < 0.0001$), while the proportion of the other colours decreased.

Similarly, the proportion of horses with white head colour showed a significant increase ($p < 0.05$) with age, while the proportion of horses with grey head colour decreased. The proportion of the others (red and red with white stripe) remained constant.

Finally, older horses also showed a higher proportion of white tail colour ($p < 0.01$) while the proportion of horses with black tail decreased. The proportion of the others (red and grey) remained the same.

The majority of the Gesha horses had a dorsal stripe and slightly convex face profile, which can be considered their unique characteristics (Table 9). A curved back profile was predominantly observed in Telo horses, which distinguished them from the others. A slight effect of sex on the qualitative characteristics was observed: shorter hair, a slightly convex face and a straight back profile were observed mainly in stallions.

Discussion

Morphometric measurements

The studied morphometric measurements produced reliable information to characterize and differentiate the three horse populations phenotypically. Besides studying the main effect (location), the effects of age and sex were also analyzed to see if they could cause a significant difference. The effect of age was not significant,

Table 9. Summary of the stepwise discriminant function analysis. Traits are listed in ascending order used in discriminating the horse populations from different locations.

Step	Variables entered	Partial R-square	F value	Pr > F	Wilks' Lambda	Pr < Lambda
1	Pelvic width	0.2214	55.60	< 0.0001	0.7785	< 0.0001
2	Cannon bone length	0.1561	36.06	< 0.0001	0.6570	< 0.0001
3	Height at croup	0.1362	30.68	< 0.0001	0.5675	< 0.0001
4	Head width	0.0888	18.91	< 0.0001	0.5171	< 0.0001
5	Body length	0.0574	11.79	< 0.0001	0.4874	< 0.0001
6	Ear length	0.0500	10.16	< 0.0001	0.4630	< 0.0001
7	Thorax depth	0.0381	7.62	0.0006	0.4454	< 0.0001
8	Shoulder depth	0.0531	10.76	< 0.0001	0.4218	< 0.0001
9	Neck body circumference	0.0393	7.84	0.0005	0.4052	< 0.0001
10	Back length	0.0308	6.07	0.0025	0.3927	< 0.0001
11	Barrel length	0.0336	6.63	0.0015	0.3795	< 0.0001
12	Thorax width	0.0272	5.32	0.0053	0.3692	< 0.0001
13	Thorax girth	0.0306	5.99	0.0028	0.3578	< 0.0001
14	Height at withers	0.0191	3.67	0.0264	0.3510	< 0.0001
15	Height at back	0.0227	4.39	0.0131	0.3430	< 0.0001
16	Neck length	0.0200	3.83	0.0225	0.3362	< 0.0001
-	Head length	0.0029	0.55	0.5754	-	-
-	Head neck circumference	0.0002	0.05	0.9555	-	-
-	Chest width	0.0028	0.52	0.5947	-	-
-	Cannon bone circumference	0.0019	0.36	0.6946	-	-
-	Croup length	0.0011	0.20	0.8210	-	-

Table 10. Values and significant levels of different statistical tests. DF, degrees of freedom.

Statistic	Value	F value	Num DF	Den DF	Pr > F
Wilk's lambda	0.3362	17.03	32	752	< 0.0001
Pillai's trace	0.8298	16.71	32	752	< 0.0001
Hotelling-Lawley trace	1.4280	17.35	32	668.29	< 0.0001
Roy's Largest Root	0.9718	22.90	16	377	< 0.0001

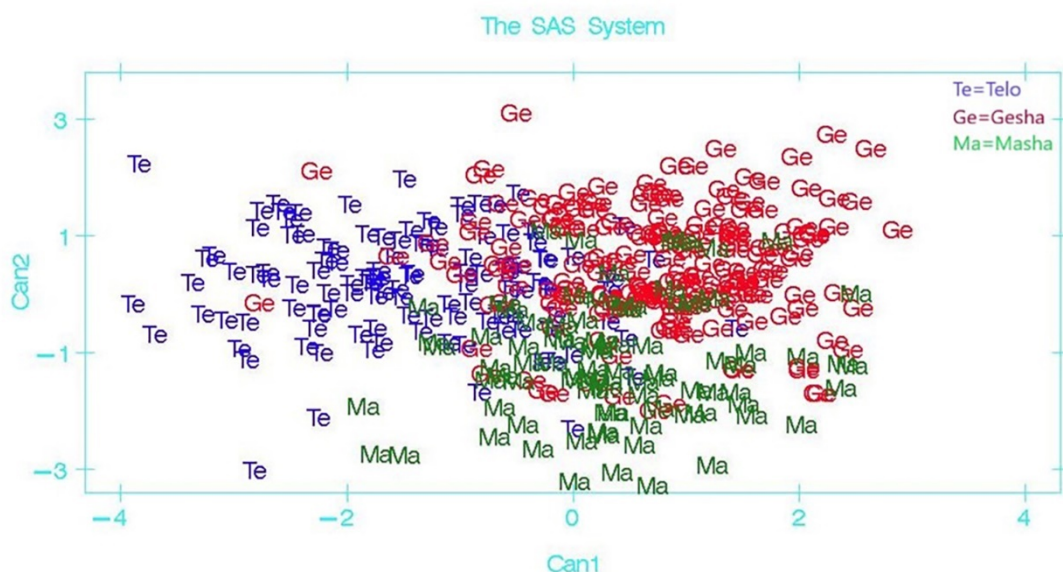


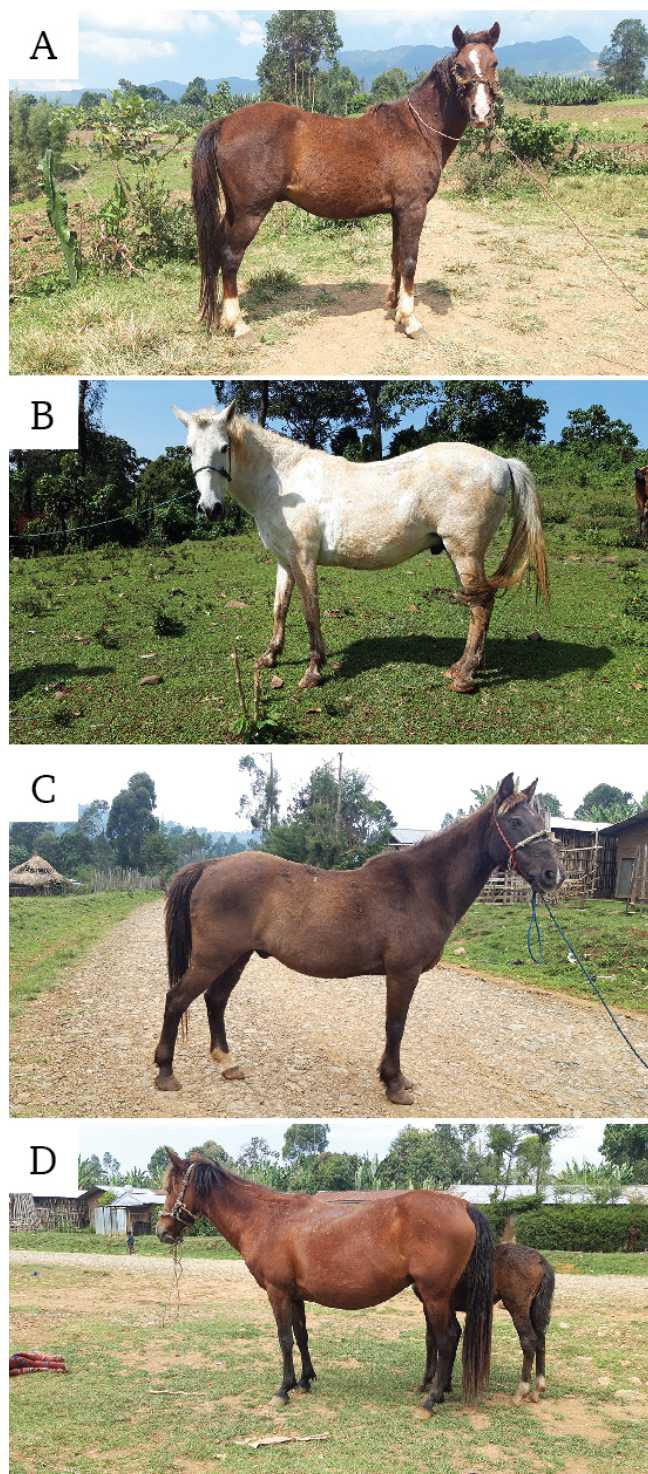
Figure 2. Plot of the first two canonical structures discriminating the three horse populations.

Table 11. Canonical correlations, eigenvalues, and class means.

	Can 1	Can 2
Multivariate Statistics		
Canonical Correlation	0.7020	0.5805
Eigenvalue	0.9718	0.5083
Proportion	0.6566	0.3434
Class (location) means		
Telo	-1.4394	0.1662
Gesha	0.7827	0.5109
Masha	0.3841	-1.2949
Raw canonical coefficients		
Head width	0.3332	-0.2810
Ear length	0.1426	-0.1680
Neck length	-0.0526	-0.0230
Neck body circumference	0.0552	0.0332
Shoulder depth	-0.1501	0.0289
Thorax depth	0.0693	-0.1063
Thorax width	0.0581	-0.1371
Thorax girth	-0.0134	0.0875
Cannon bone length	-0.3522	-0.1375
Height at withers	-0.1633	-0.0871
Height at back	0.1627	-0.0541
Height at croup	0.0777	0.2509
Body length	-0.0567	0.0267
Back length	-0.0606	0.0513
Pelvic width	0.1924	-0.0206
Barrel length	0.0350	-0.0723

which might be due to the nature of the sampling, which included adult horses only. On the other hand, sex significantly affected the studied traits. Stallions had higher values than mares on most morphometric measurements, in line with Rensch's rule (Rensch, 1950). According to Rensch (1950), males of a given species are usually larger than females. Such differences between stallions and mares may be ascribed to levels of testosterone secreted by stallions, which leads to larger muscle mass and skeletal development (Baneh and Hafezian, 2009). Similar results were also reported by Kefena *et al* (2012), Ghezelsoflou *et al* (2018) and Sadek *et al* (2006) on Ethiopian, Iranian Turkoman and Arabian horses, respectively.

According to Kefena *et al* (2012), Selale horses (the tallest and typical riding horses in Ethiopia) had values of 131.2 ± 0.4 , 125.6 ± 0.4 , and 131.7 ± 0.5 cm for heights at withers, back and croup, respectively. The current study revealed that Gesha horses are the tallest horses in Ethiopia with a value of 132.8 ± 0.37 , 130.4 ± 0.36 , and 134.0 ± 0.36 cm for heights at withers, back and croup, respectively (Table 6). However, these values were much lower than the reports of Zechner *et al* (2001) for Lipizzan horses studied in different locations in Europe, and Ghezelsoflou *et al* (2018) for Iranian Turkoman horses in Iran. The tall and big body of the Gesha horse population in Ethiopia indicates that they

**Figure 3.** A, Telo stallion; B, Masha stallion; C, Gesha stallion; D, Gesha mare. Photo: Amine Mustefa, EBI

can be categorized as typical saddle horses. This is in line with the study by Kristjansson *et al* (2016) in Iceland, which showed a higher riding ability as the horses' height increased. Traditionally, Gesha horses, which are known for their aggressiveness, are also known and recognized as typical riding horses.

The barrel and neck lengths, and cannon bone length and circumference for all the populations from the current study are comparable with the reports of Kefena *et al* (2012) on all Ethiopian horse populations. The

Table 12. Number and percentage of observations classified into locations.

From location	Telo	Gesha	Masha	Total
Telo	98 (79.7%)	14 (11.4%)	11 (8.9%)	123 (100%)
Gesha	19 (10.4%)	140 (76.5%)	24 (13.1%)	183 (100%)
Masha	7 (7.9%)	16 (18.2%)	65 (73.9%)	88 (100%)
Total	124 (31.5%)	170 (43.1%)	100 (25.4%)	394 (100%)
Error rate	0.203	0.235	0.261	0.233
Priors	0.333	0.333	0.333	

Table 13. Squared Mahalanobis distance between locations; output of the multivariate analysis calculated using the quantitative measurements. *** shows the significance of the distance calculations at $p < 0.0001$.

From location	Telo	Gesha	Masha
Telo	0		
Gesha	5.06***	0	
Masha	5.46***	3.42***	0

body length of Gesha horses ($127.0 \pm 0.49\text{cm}$) is lower than the reports of Kefena et al (2012) for all Ethiopian horse populations. On the other hand, the head and back lengths of Gesha horses (53.6 ± 0.20 and $71.2 \pm 0.33\text{cm}$, respectively) is higher than all Ethiopian horse populations (Kefena et al, 2012). Such wide disagreement might be due to differences in points of measurement. The thorax girth of Gesha horses ($146.6 \pm 0.56\text{cm}$) is comparable with Selale ($146.6 \pm 0.8\text{cm}$), Bale ($145.3 \pm 0.7\text{cm}$), and Horro horses ($145.5 \pm 0.6\text{cm}$) while it was higher than Abyssinian horses ($140.4 \pm 0.5\text{cm}$) and lower than Keffa horses ($152.6 \pm 0.7\text{cm}$) (Kefena et al, 2012).

Body measure indices

The body index shows the length of the animal. A long animal is best suited for speed, a short animal for strength (Torres and Jardim, 1981). Long animals have a body index value greater than 90, while a value less than 85 indicates that the animal is short (Torres and Jardim, 1981). According to Table 8, the Telo and Masha mares were categorized as long horses. However, in reality, Gesha stallions are known for their speed.

The caliber index, which shows the overall size of the horse, increases with age and size (Kaps et al, 2005). Kaps et al (2005) observed its increase from 119.1 to 135 in Lipizzan horses from 6 to 36 months of age. The current findings show the comparably big size of Gesha stallions.

The overbuilt index of a horse indicates the proportion of its height at withers and at croup. A horse with downhill conformation (height at croup higher than height at withers) is indicated as the best riding horse by Padilha et al (2017), since stronger muscles in the hind limbs and taller hind limbs indicate greater power for jumping and the ability to give a solo performance. In line with the current findings, Mcmanus et al (2005) in Campeiro horses, Rezende et al (2014) in Brazilian sport horses and Mariz et al (2015) in Quarter horses reported a slightly downhill conformation. However, uphill conformation was reported as an important characteristic by Lucena et al (2015) in Marchador horses and Kristjansson et al (2016) in Icelandic horses.

According to Torres and Jardim (1981), a riding horse must have a conformation index value of 2.1125. A value above this threshold shows the suitability of a horse for work. The conformation index values found in the current study were between 1.47 and 1.65 (Table 8),

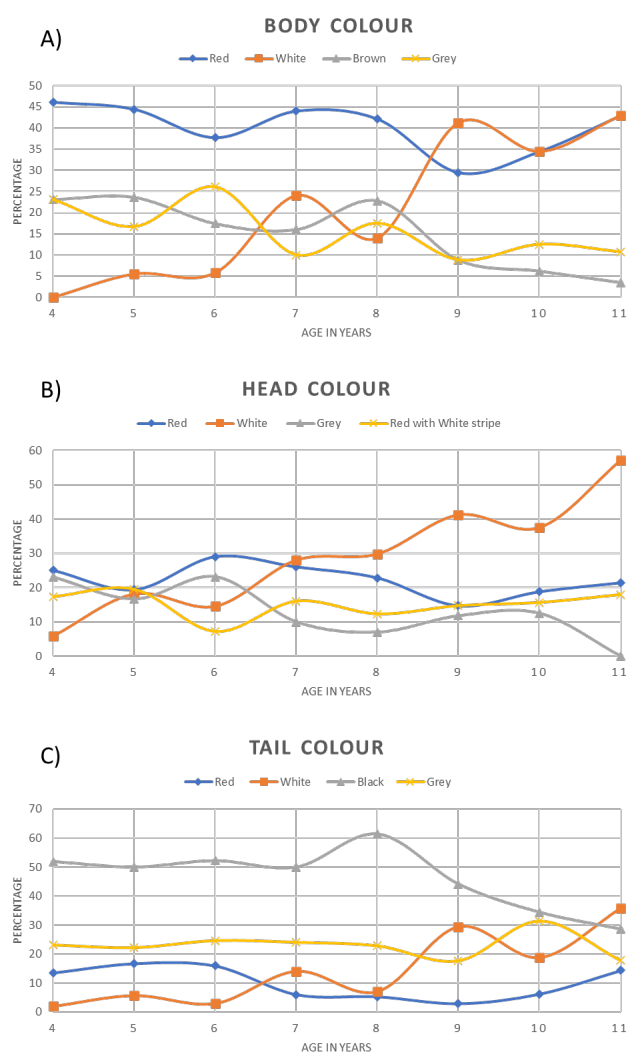
**Figure 4.** Effect of age on colour characteristics of horse populations. A) Body colour; B) Head colour; C) Tail colour.

Table 14. Statistical values for chi-square and Cramér's V, and level of significance (probabilities) for the effects of location, sex and age on the qualitative characteristics of the studied horse populations: aggregate sex. χ^2 , chi-square; prob., probabilities; *, < 0.05; **, < 0.01; ***, < 0.0001; NS, Not significant.

Qualitative traits	Location			Sex			Age		
	χ^2 value	Cramér's V	Prob.	χ^2 value	Cramér's V	Prob.	χ^2 value	Cramér's V	Prob.
Body colour	43.1	0.234	***	6.4	0.127	NS	95.2	0.201	***
Head colour	34.8	0.210	*	19.4	0.222	*	90.5	0.432	*
Muzzle colour	37.1	0.217	***	5.9	0.122	NS	42.2	0.164	*
Tail colour	23.7	0.173	**	9.6	0.156	*	58.1	0.192	**
Hoof colour	55.8	0.266	***	1.9	0.069	NS	38.2	0.220	**
Hair size	21.3	0.233	***	12.4	0.178	**	9.7	0.157	NS
Body colour pattern	8.7	0.105	NS	0.07	0.014	NS	8.3	0.103	NS
Dorsal stripe	16.5	0.205	**	0.2	0.021	NS	10.5	0.163	NS
Shoulder stripe	1.8	0.068	NS	1.6	0.064	NS	4.8	0.111	NS
Face profile	52.9	0.367	***	4.1	0.102	*	4.3	0.105	NS
Back profile	52.8	0.366	***	4.0	0.101	*	2.6	0.081	NS
Tail length	28.4	0.190	***	4.2	0.103	NS	17.4	0.149	NS
Mane length	52.8	0.259	***	2.5	0.080	NS	10.0	0.112	NS

Table 15. Percentages of colour-related qualitative traits of the horses (both sexes) from different locations.

Colour-related qualitative traits		Location			Sex	
		Telo	Gesha	Masha	Stallions	Mares
Body colour	Red	30.1	50.8	35.2	42.2	37.5
	Brown	20.3	13.1	21.6	14.5	24.1
	Gray	16.3	15.8	20.5	18.1	14.3
	White	20.3	13.1	18.2	17.4	14.3
	Tan	0.8	5.5	4.6	3.5	4.5
	Black	9.8	1.1	0.0	3.2	4.4
	Red and white	2.4	0.6	0.0	1.1	0.9
Head colour	White	30.9	19.7	28.4	26.9	20.5
	Gray	18.7	12.0	13.6	15.3	12.5
	Red	21.1	26.2	18.2	24.5	18.8
	Red with white stripe	5.7	21.9	12.5	14.9	14.3
	Black	14.6	9.8	9.1	10.3	13.4
	Black with white stripe	0.8	1.6	4.5	1.8	2.7
	Brown	5.7	4.9	9.1	3.5	12.5
Muzzle colour	Brown with white stripe	1.6	0.6	1.1	0.7	1.8
	Tan	0.8	1.1	0.0	1.1	0.0
	Tan with white stripe	0.0	2.2	3.4	1.1	3.6
	Black	51.2	36.6	37.5	39.4	46.4
	White	26.8	19.7	12.5	22.7	14.3
	Red	10.6	25.7	18.2	19.1	10.6
	Gray	11.4	9.3	21.6	13.5	10.7
Tail colour	White and Black	0.0	8.7	10.2	5.3	8.9
	Black	52.0	53.0	36.4	48.6	50.0
	Gray	26.0	19.7	26.1	25.2	17.9
	White	13.8	8.7	12.5	11.7	9.8
	Red	4.9	14.8	12.5	11.0	11.6
	Brown	3.3	3.8	12.5	3.5	10.7
	Black	91.9	74.9	52.3	74.1	77.7
Hoof colour	Black and White	4.9	21.8	47.7	22.7	21.4
	White	3.2	3.3	0.0	3.2	0.9

Table 16. Percentages of qualitative traits of the horses (both sexes) from different locations.

Qualitative traits		Location			Sex	
		Telo	Gesha	Masha	Stallions	Mares
Hair size	Short	42.3	43.2	15.9	42.2	23.2
	Medium	57.7	56.8	84.1	57.8	76.8
Body colour pattern	Plain	95.9	99.4	100	98.6	98.2
	Pied	1.6	0.6	0.0	0.7	0.9
	Shaded	2.4	0.0	0.0	0.7	0.9
Dorsal stripe	Absent	67.5	44.3	57.9	53.9	56.3
	Present	32.5	55.7	42.1	46.1	43.7
Shoulder stripe	Absent	99.2	99.4	97.7	98.6	100
	Present	0.2	0.6	2.3	1.4	0.0
Face profile	Straight	86.2	45.4	65.9	59.6	70.5
	Slightly convex	13.8	54.6	34.1	40.4	29.5
Back profile	Straight	44.7	76.5	87.5	72.0	61.6
	Curved	55.3	23.5	12.5	28.0	38.4
Tail length	Short	2.4	0.0	0.0	1.1	0.0
	Medium	40.7	22.4	14.8	28.7	20.5
	Long	56.9	77.6	85.2	70.2	79.5
Mane length	Short	4.9	0.0	0.0	2.1	0.0
	Medium	48.0	16.9	39.8	31.9	31.3
	Long	47.1	83.1	60.2	66.0	68.7

with Gesha stallions having the highest conformation index values among the studied populations.

Multivariate analysis

Stepwise discriminant function analysis selected and ranked the morphometric variables according to their importance in discriminating the studied horse populations. The inclusion of height at croup and body length within the top five discriminatory variables is comparable with the reports of Kefena et al (2012), who classified them among the top four variables to discriminate Ethiopian horse populations. The results of discriminant function analysis showed an advanced classification (76.7%) of the studied horses into their respective populations/locations. This high value shows the dissimilarity among the studied populations. Canonical discriminant function analysis revealed the higher power of Can 1 than Can 2 to separate the horse populations. This shows the separation of Gesha and Masha horses from Telo horses while differences also occur between Gesha and Masha populations. However, the distances showed only the relative size differences between each population. Such differences might not necessarily be due to breed (genetic) differences (Zechner et al, 2001). Therefore, a diversity study through further genetic characterization is recommended to design conservation and breeding programmes.

Qualitative characteristics

Besides their aggressiveness and top-riding ability, the examined qualitative characteristics clearly differentiated the Gesha horse population from the other studied populations. The majority of Gesha horses possess

red body colour, red and white-striped red head colour, striped dorsal body, slightly convex face and long mane while some similarities were observed with the adjacent Masha horses. A slight effect of sex and age on the qualitative characteristics was observed. Shorter hair, a slightly convex face and a straight-back profile were observed predominantly in stallions than mares.

The current study revealed the level of relationship between age and body colour. As age advanced, the proportion of horses with white (body, head and tail) colour increased while the proportion of horses with grey and brown colours decrease, which might be due to the progressive depigmentation of the coat's hairs (Locke et al, 2002). At birth, grey horses may have any colour but over time, white hairs begin to appear and become gradually more dominant as white hairs become intermixed with hairs of other colours. At a later age, most horses of this type ultimately become completely white, though some retain intermixed light and dark hairs (Locke et al, 2002). This is due to the presence of a greying allele of the KIT gene, which inhibits the hair follicles from producing melanin. The coat takes on a 'dappled' pattern that increasingly becomes white. However, grey horses with a totally white coat can be distinguished from white horses by their underlying black skin, particularly around the eyes, muzzle, and genital area (Locke et al, 2002).

Conclusion

The studied phenotypic traits (morphometric measurements and qualitative characteristics) had produced reliable information in characterizing and differentiating Gesha, Masha and Telo horse populations. Gesha horses

were the tallest, longest and largest among the studied horse populations. Besides their size, the most important characteristics of Gesha horses are their aggressiveness, top-riding ability, red-dominated body colour, white-striped red head colour and slightly convex face. These results were also supported by the multivariate analysis, which differentiated the Gesha horse population from the Masha and Telo horse populations, and showed a relatively higher relationship with Masha horses. Further genetic characterization is recommended to confirm the above results and design conservation and breeding programmes.

Acknowledgments

The authors are highly indebted to the Ethiopian Biodiversity Institute (EBI) for covering all the budget needs of the work. Our special appreciation also goes to the animal owners for providing their animals for this work for free. We also take this opportunity to thank the animal science experts and development agents in the districts for their endless help during data collection. A special word also goes to our friend and work partner Mr Tadesse Hunduma for mapping the study area.

Author contributions

All authors contributed to the study conception and design. Material preparation and data collection were performed by Amine Mustefa, Aweke Engdawork, and Seble Sinke. Amine Mustefa performed the data analysis and wrote the first draft of the manuscript. All authors commented on previous versions of the manuscript, and read and approved the final manuscript.

Conflict of interest statement

The authors declare that they have no conflict of interest.

References

- Assefa, A., Demissew, S., and Woldu, Z. (2013). Floristic composition, structure and regeneration status of Masha forest, south-west Ethiopia. *African Journal of Ecology* 52, 151–162. doi: <https://doi.org/10.1111/aje.12098>
- Baneh, H. and Hafezian, S. H. (2009). Effect of environmental factor on growth traits in Ghezel sheep. *African Journal of Biotechnology* 8, 2903–2907. doi: <https://www.ajol.info/index.php/ajb/article/view/60943>
- Bene, S., Gicz, A., Nagy, Z., Benedek, Z., Szabó, F., and Polgár, J. P. (2013). Live Weight and Body Measurement of Hungarian Thoroughbred Broodmares. *Journal of Central European Agriculture* 14(3), 99–109. doi: <https://doi.org/10.5513/JCEA01/14.3.1287>
- Bezabih, T. (2012). Study on the intensity and adoption of improved wheat varieties and associated agronomic practices in Kaffa zone, the case of Gesha woreda. MSc thesis, Indira Gandhi National Open University, India.
- Bodó, I. and Hecker, W. (1992). Handbook of Horse Breeders (Budapest, Hungary: Mezőgazda Publisher).
- Cabral, G. C., De Almeida, F. Q., Quirino, C. R., De Azevedo, P. C. N., Pinto, L. F. B., and Santos, E. M. (2004). Morphometric evaluation of Mangalarga Marchador horse: Conformation index and body proportions. *Revista Brasileira de Zootecnia* 33, 1798–1805. url: https://www.researchgate.net/publication/262481742_Morphometric_evaluation_of_Mangalarga_Marchador_horse_Conformation_index_and_body_proportions.
- Central Statistical Agency (2020). Ethiopian agricultural sample survey 2019/2020 (2013 E. C.). In Report on Livestock and Livestock Characteristics, volume II, Central Statistical Agency, Addis Ababa, Ethiopia, 20–20.
- Druml, T., Baumung, R., and Sölkner, J. (2008). Morphological analysis and effect of selection for conformation in the Noriker draught horse population. *Livestock Science* 115, 118–128. doi: <https://doi.org/10.1016/j.livsci.2007.06.015>
- EBI (2016). Ethiopian National Strategy and Plan of Action for conservation and utilization of Animal Genetic Resources.
- FAO (2012). Phenotypic Characterization of Animal Genetic Resources. url: www.fao.org/docrep/015/i2686e/i2686e00.pdf.
- Gebremichael, A. (2019). Cattle milk production, processing and marketing situations of smallholder farmers in Telo district, Keffa zone, Ethiopia. *African Journal of Agricultural Research* 14(18), 806–812. url: <https://academicjournals.org/journal/AJAR/article-full-text-pdf/3270D8460881>.
- Ghezelsoflou, H., Hamidi, P., and Gharahveysi, S. (2018). Study of factors affecting the body conformation traits of Iranian Turkoman horses. *Journal of Equine Science* 29(4), 91–96. doi: <https://doi.org/10.1294/jes.29.91>
- Kaps, M., Curik, I., and Baban, M. (2005). Genetic Analysis of the Caliber Index in Lipizzan Horses Using Random Regression. In 56th Annual Meeting of the European Association for Animal Production (EAAP).
- Kefena, E., Dessie, T., Han, J. L., Kurtu, M. Y., Rosenbom, S., and Beja-Pereira, A. (2012). Morphological diversities and eco-zones of Ethiopian horse populations. *Animal Genetic Resources* 50, 1–12. doi: <https://doi.org/10.1017/S2078633612000021>
- Kristjánsson, T., Björnsdóttir, S., Albertsdóttir, E., Sigurdsson, A., Pourcelot, P., Crevier-Denoix, N., and Arnason, T. (2016). Association of conformation and riding ability in Icelandic horses. *Livestock Science* 189, 91–101. doi: <https://doi.org/10.1016/j.livsci.2016.05.010>
- Locke, M. M., Penedo, M. C. T., Bricker, S. J., Millon, L. V., and Murray, J. D. (2002). Linkage of the grey coat color locus to microsatellites on horse chromosome 25. *Animal Genetics* 33, 329–337. doi: <https://doi.org/10.1046/j.1365-2052.2002.00885.x>

- Lucena, J. E. C., Vianna, S. A. B., Neto, F., Filho, R. L. M. S., and Diniz, W. J. S. (2015). *Estudo comparativo das proporções morfométricas entre garanhões e castrados da raça Campolina* 36, 353–366. doi: <http://dx.doi.org/10.5433/1679-0359.2015v36n1p353>
- Mariz, T. M. A., Santos, W. K., Mota, L. F. M., Martins, R. B., Lima, C. B., Escodro, P. B., Júnior, D. M. L., Oliveira, L. P., Sousa, M. F., and Ribeiro, J. S. (2015). Avaliação de medidas morfoestruturais em equinos da raça Quarto de Milha utilizando análises de imagens. *Acta Veterinaria Brasilica* 9, 362–368. url: [https://www.bvs-vet.org.br/vetindex/periodicos/acta-veterinaria-brasilica/9-\(2015\)-4/avaliacao-de-medidas-morfoestruturais-em-equinos-da-raca-quarto-de-mil/](https://www.bvs-vet.org.br/vetindex/periodicos/acta-veterinaria-brasilica/9-(2015)-4/avaliacao-de-medidas-morfoestruturais-em-equinos-da-raca-quarto-de-mil/).
- Mcmanus, C., Falcão, R. A., Spritze, A., Costa, D., Louvandini, H., Dias, L. T., Teixeira, R. A., Rezende, M. J. M., and Garcia, J. A. S. (2005). Caracterização morfológica de equinos da raça Campeiro. *Revista Brasileira de Zootecnia* 34, 1553–1562. url: <https://repositorio.unb.br/handle/10482/6858>.
- Padilha, F. G. F., Andrade, A. M., Fonseca, A. B. M., Godoi, F. N., Almeida, F. Q., and Ferreira, A. M. R. (2017). Morphometric measurements and animal-performance indices in a study of racial forms of Brazilian Sport Horses undergoing training for eventing. *Revista Brasileira de Zootecnia* 46(1), 25–32. doi: <https://doi.org/10.1590/S1806-92902017000100005>
- Rensch, B. (1950). Die Abhängigkeit der relative sexual differenz von der korpergrosse. *Bonner Zoologische Beitrage* 1, 58–69. url: <https://www.biodiversitylibrary.org/partpdf/119381>.
- Rezende, M. P. G., Souza, J. C., Mota, M. F., Jardim, R. J. D., Ramires, G. G., Silva, R. M., and Souza, C. F. (2014). Morfometria corporal de equinos utilizados em trabalho, esporte e lazer em três municípios do Mato Grosso do Sul. *Veterinária e Zootecnia* 21, 569–583. url: https://www.researchgate.net/publication/284178649_MORFOMETRIA_CORPORAL_DE_EQUINOS_UTILIZADOS_EM_TRABALHO_ESPORTE_E_LAZER_EM_TRES_MUNICIPIOS_DO_MATO_GROSSO_DO_SUL_MORPHOMETRY_BODY_OF_EQUINES_USED_IN_WORK_SPORT_AND_LEISURE_IN_THREE_CITIES_OF_MATO_GROSSO.
- Sadek, M. H., Al-Aboud, A. Z., and Ashmawy, A. A. (2006). Factor analysis of body measurements in Arabian horses. *Journal of Animal Breeding and Genetics* 123, 369–377. doi: <https://doi.org/10.1111/j.1439-0388.2006.00618.x>
- SAS Institute (2002). Statistical Analysis Software (SAS). url: https://www.sas.com/en_us/home.html.
- Torres, A. P. and Jardim, W. R. (1981). Criação do cavalo e de outros eqüinos. Nobel, São Paulo.
- Zechner, P., Zohman, F., Solkner, J., Bodo, I., Habe, F., Marti, E., and Brem, G. (2001). Morphological description of the Lipizzan horse population. *Livestock Production Science* 69, 163–177. doi: [https://doi.org/10.1016/S0301-6226\(00\)00254-2](https://doi.org/10.1016/S0301-6226(00)00254-2)



Survey and conservation of crop landraces in northwest Syria

Munzer Al Darvish ^{*,a}, Anas Al Kaddour ^b, Akram Bourgol ^c, Yasser Ramazan ^d, Yousef Hallak ^e and Shelagh Kell ^f

^a Aleppo University and Agriculture Research Centre, Syria

^b Aleppo University and General Organization for Seed Multiplication, Syria

^c General Commission for Scientific Agricultural Research, Syria

^d Plant Protection Directorate, Ministry of Agriculture, Syria

^e Ministry of Agriculture, Syria

^f School of Biosciences, University of Birmingham, Edgbaston, B15 2TT, Birmingham

Abstract: Syria lies at the heart of the Fertile Crescent – one of the centres of diversity of staple crops such as wheat, barley, chickpea and lentil. The country has historically been rich in agrobiodiversity, including crop landraces valued for their nutritional and culinary qualities, as well as for their resilience. With their cultivation already in decline before the start of the Syrian crisis in 2011, this study was undertaken to assess the current status of crop landraces in northwest Syria, and to initiate an *ex situ* conservation programme. We found a significant decline in the number of landraces in cultivation, indicating a loss of locally adapted genetic diversity. Influencing factors include insufficient seed supply, competition with commercial hybrids, falling market demand and neglect by relevant government authorities. Despite not seeing conservation as their responsibility, the participating farmers were supportive of landrace conservation and willingly contributed seeds for *ex situ* conservation.

Keywords: Plant genetic resources, Genetic erosion, On-farm conservation, Landraces

Citation: Al Darvish, M., Al Kaddour, A., Bourgol, A., Ramazan, Y., Hallak, Y., Kell, S. (2022). Survey and conservation of crop landraces in northwest Syria. *Genetic Resources* 3 (5), 51–58. doi: [10.46265/genresj.USTS1801](https://doi.org/10.46265/genresj.USTS1801).

© Copyright 2022 the Authors.

This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

Crop landraces have been defined as “dynamic populations” of cultivated plant species that have distinct identities and historical origins, and which lack formal crop improvement, and are typically “genetically diverse, locally adapted and associated with traditional farming systems” (Camacho-Villa *et al*, 2005). They are “closely associated with the uses, knowledge, habits, dialects, and celebrations of the people who developed and continue to grow” them (Veteläinen *et al*, 2008), and their continued existence traditionally relies on repeated cycles of seed selection and sowing (Almekinders *et al*, 1994; Maxted *et al*, 1997). These populations character-

istically exhibit high levels of adaptation to local environmental conditions, including abiotic and biotic stress tolerances, through a combined process of human and natural selection (Almekinders *et al*, 1994).

Crop landraces are valued not only for their adaptation and resilience to local climatic conditions and resistance to pests and diseases, but also for their nutritional and culinary qualities – such as flavour, colour and texture – and their cultural value (FAO, 2019b). Furthermore, they constitute a valuable pool of genetic diversity for crop improvement – particularly in the development of varieties with abiotic and biotic stress tolerance and for incorporating farmer-preferred traits (FAO, 2019b). However, the socioeconomic change and transformation of production systems that occurred worldwide in the twentieth century, has resulted in the substitution of landraces with modern cultivars and a

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

widespread reliance on crop monocultures (Veteläinen *et al*, 2008; Van De Wouw *et al*, 2010; Frison *et al*, 2011; Maxted *et al*, 2011; Dwivedi *et al*, 2016; FAO, 2019b; Petropoulos *et al*, 2019). This homogenization, and associated loss of landrace diversity, has rendered agriculture, food security and livelihoods vulnerable to the impacts of climate change, such as drought, heatwaves, storms, floods and frosts, as well as unexpected pest and disease outbreaks (GenRes Bridge Project Consortium, ECPGR, ERFP and EUFORGEN, 2021).

Under the current climate crisis, diversification in farming is needed, including the use of more genetically diverse crop varieties resilient to the increasingly extreme and uncertain impacts of climate change (FAO, 2008, 2019a; GenRes Bridge Project Consortium, ECPGR, ERFP and EUFORGEN, 2021). The conservation of crop landraces is therefore an essential component of sustainable agricultural development (Dwivedi *et al*, 2016; Ficiciyan *et al*, 2018; FAO, 2019b), and this includes incentives to retain the cultivation of landraces on farm, as well as to ensure complementary *ex situ* conservation in genebanks as a backup for potential losses in farmers' fields, and for access for research and crop improvement (Maxted *et al*, 1997, 2011; Veteläinen *et al*, 2008; FAO, 2011, 2019b).

Syria lies in the Fertile Crescent – a region of considerable topographic and climatic diversity (MSEA, 2009) and in the historical centre of origin of many world food crop staples, such as cereals (e.g. wheat, barley and rye), legumes (e.g. lentil, chickpea and broad bean) and fruits and nuts (e.g. peach, pear, almond and pistachio), as well as animal feed (Vavilov, 1926; Damania, 1994; FAO, 1995; Willcox, 2012; Mazid *et al*, 2014; Jaradat, 2017). Syrian crop landrace diversity has contributed to crop improvement for increased agricultural production (e.g. in barley (Ceccarelli and Grando, 2000)). The same authors concluded that “securing the continuity of the evolutionary processes within landrace populations is of vital importance for future generations.”

The threat to Syrian crop landraces was already recognized in the 1990s, particularly in relation to wheat, vegetables and fruit trees, as a result of the adoption of genetically uniform modern varieties and the lack of policies to protect landraces from competing commercial varieties (FAO, 1995). Although landraces remain in cultivation in Syria, their use has declined over time due to the push towards new higher-yielding varieties, when fertilizers and agrochemicals are available (FAO, 1995). In addition, the younger generation is moving away from agriculture, either as a profession or for subsistence purposes (Veteläinen *et al*, 2008; Maxted *et al*, 2011; Mazid *et al*, 2014), and while crop landraces are often valued for their unique qualities, some do not produce sufficiently high yields for adequate income generation (Veteläinen *et al*, 2008).

Organizations active in plant genetic resources conservation and crop research in Syria before the onset

of the crisis in 2011 included the Arab Centre for the Study of Arid Zones and Dry Lands (ACSAD), founded in Damascus in 1968, the Syrian Ministry of Agriculture and Agrarian Reform (SMAAR) seedbank in Douma, and the International Centre for Agricultural Research in the Dry Areas (ICARDA), established in Tel Hadya in 1975. The majority of ICARDA's international staff fled Syria in 2012, with its genebank operations ceasing in 2015 (Alkiswany, 2012; Gewin, 2015). Following damage to its facilities, the General Organization for Seed Multiplication (GOSM), the national source of seed multiplication and supply for commercial varieties, also ceased its operations in Idleb Governorate, northwest (NW) Syria (CRS, 2015). In 2013, the Syrian Interim Government (SIG) established a parallel seed-multiplication entity in opposition-controlled areas of Idleb and Aleppo Governorates to try to meet local needs in NW Syria through the import and export of commercial/hybrid seeds for cereals, legumes, potatoes, and vegetables, with its seed-multiplication activities limited to commercial wheat varieties.

Since the beginning of the Syrian crisis, agricultural production has fallen considerably due to drought, migration, insecurity, loss of government subsidies and the rising prices of inputs, such as fuels and fertilizers, as well as limited access to markets (Kelley *et al*, 2015; Mohammed *et al*, 2019; Al-Ghazi, 2021). For example, by 2017, only 18 out of 33 commercial wheat varieties were still in use by farmers in NW Syria, 9 for durum and 9 for soft wheat (GOSM, 2017). At the same time, the conservation of Syria's crop landraces has not been a priority for local authorities and humanitarian actors, and initiatives to conserve local genetic resources have been limited by insecurity and loss of expertise. At national level, ACU (2016) reported that Syria's farmers were still producing 32% of their own wheat seeds, while being reliant on traders for 50%, and other sources (including donated and subsidized seed) for the remaining 18%. However, in NW Syria, farmers were reported to source 90–95% of their seed through informal channels (CRS, 2015). Further, the primary focus of ICARDA and SMAAR on cereals and cash crops has resulted in an absence of data on landrace vegetables, legumes (e.g. common bean and cowpea) and forage crop varieties (ICARDA, 2017).

It was within this context that in 2018, the authors initiated a study of the status of crop landraces of vegetables, legumes, cereals, forages and cash crops in NW Syria, with the aim of ensuring the availability of this diversity for future generations, and in the longer term, the multiplication and provision of good quality seeds for small-scale farmers in the region. Specifically, the objectives were to: (a) understand the current use of crop landraces in NW Syria and assess whether there has been any loss of diversity, (b) reveal the factors influencing their continued cultivation as well as the potential causes for declining use and (c) inform the process of collecting landrace seeds to initiate *ex situ* conservation.

Materials and methods

The study was conducted between December 2018 and November 2019 in Idleb Governorate in the districts of Maaret Tamisrin, Saraqeb, Ma'arrat An'Numan and the Idleb subdistrict (Figure 1). This region is located within the second and third agroecological zones, incorporating both mountains and plains. Agroecological zone 2 covers 2,473,000 ha (13.4% of the country) with an annual rainfall of 250 to 350mm and no less than 250mm across two-thirds of the monitored years. Agroecological zone 3 comprises 1,306,000 ha (7.1% of the country) with an annual rainfall of 250 to 350mm and no less than 250mm over half of the monitored years (FAO, 2003).

In December 2018, prior to starting the data collection, preliminary meetings were held with local council and community representatives in each of the four districts to explain the information on the study, its methods, objectives and importance, as well as to help identify suitable study participants from the local areas (i.e. individuals with considerable experience and a good knowledge of agriculture and crop diversity within the study area).

The study combined 75 one-to-one face-to-face interviews and 25 focus groups (FGs), each of which involved 5 to 10 participants. The research participants included established farmers (67), agricultural engineers (15) and agricultural researchers (18) – the latter including former local researchers from ICARDA and the General Commission for Scientific Agricultural Research (GCSAR). An interview questionnaire (see Supplemental Data) was developed and shared with both interviewees and FG participants, requiring them to answer questions as to which landraces had fallen out of use and why they believed this had happened, as well as to provide related timeframes. In the FGs, each of the questions was discussed collectively, in order to reach consensus on the answer.

The recruitment of participants with good local background knowledge was central to obtaining a full and accurate picture and to achieving the research objectives, as was the willingness of study participants to share that local knowledge. The majority of the farmers involved (89%) had farms of 1,000m² plus, so of sufficient size to be cultivating a range of crops, with 22 of those interviewed aged 55 years or over. The age and experience of this group provided the study with good historical knowledge of the crop landraces that had been cultivated in the area, as well as changes in their use. Due to local social norms, all study participants were male. However, some farmers' wives participated in the interviews and shared their knowledge.

The one-to-one interviews were conducted from January to March 2019 using hard copies of the questionnaire to allow interviewees to provide answers in writing. As an introduction to the interviews, the interviewer shared information on the study, its methods, objectives and anticipated duration. Participants were then invited to sign a consent form. If the participant was happy to proceed, the interviewer then proceeded to

the interview, reading out each question in sequence. Where requested, the interviewer provided further clarification to avoid any misunderstanding and ensure accurate responses to the questions. Answers were provided by the interviewees in writing on the questionnaire form. In the case of illiteracy amongst some of the farmers, the interviewer wrote interviewee responses on their behalf. In the case of the FGs, the interviewer captured the groups' answers in writing once consensus had been reached. All interview and FG data were transferred to MS Excel and anonymized by the team prior to analysis, to ensure confidentiality. The research team discussed the characteristics of each landrace to make sure that each was correctly identified and recorded in one of the five crop categories (vegetables, legumes, cereals, forages and cash crops).

The study also involved asking participating farmers to share seed samples of particular landraces to form the nucleus of the *ex situ* collection. Seed samples from 74 landraces, both field crops and vegetables, were collected from farmers across the four districts and planted for multiplication purposes in a safe location in Sarmada, NW Syria, near the Syrian-Turkish Bab Alhawa border crossing, using typical local cultural practices. At maturity, seeds were collected, dried and disinfected using thiram fungicide. Each sample was then placed in a labelled paper bag on which the variety, original location, source of the variety, name of the farmer who had donated the sample (with their permission), sample size, collection date and storage date were recorded, prior to being stored in airtight plastic barrels with dry silica gel. At this stage, this modest seed-multiplication activity aims only to increase their quantity as part of this *ex situ* collection. Additional funds will be required to expand seed-multiplication activities in order to support their distribution for use by local farmers.

Results

The 75 interviewees and 25 FG participants took part willingly in the study, providing the information being sought by the study team. They had good knowledge of the crop landraces that had been widely cultivated in their local area over the previous 20 years, and which were still in cultivation in 2019. More than half of the 73 landraces recorded in the study as previously widely cultivated were of vegetable crops (54%) – the remaining comprising legumes (19%), cereals (18%), forages (7%), and cash crops (2%) (Table 1).

In terms of actual numbers (Table 1), the study revealed that amongst the ten wheat landraces that were once widely cultivated, only five were still in use, and of the five species of legumes being grown in the study area, the number of landraces that were still in use had fallen from 23 to 11. The eight vegetable crop species had once included 27 landraces, whereas only 14 were still in widespread cultivation in 2019. Of the four sunflower cash-crop landraces known to the farmers, only one was no longer grown, whereas the majority of forage landraces (six out of nine)

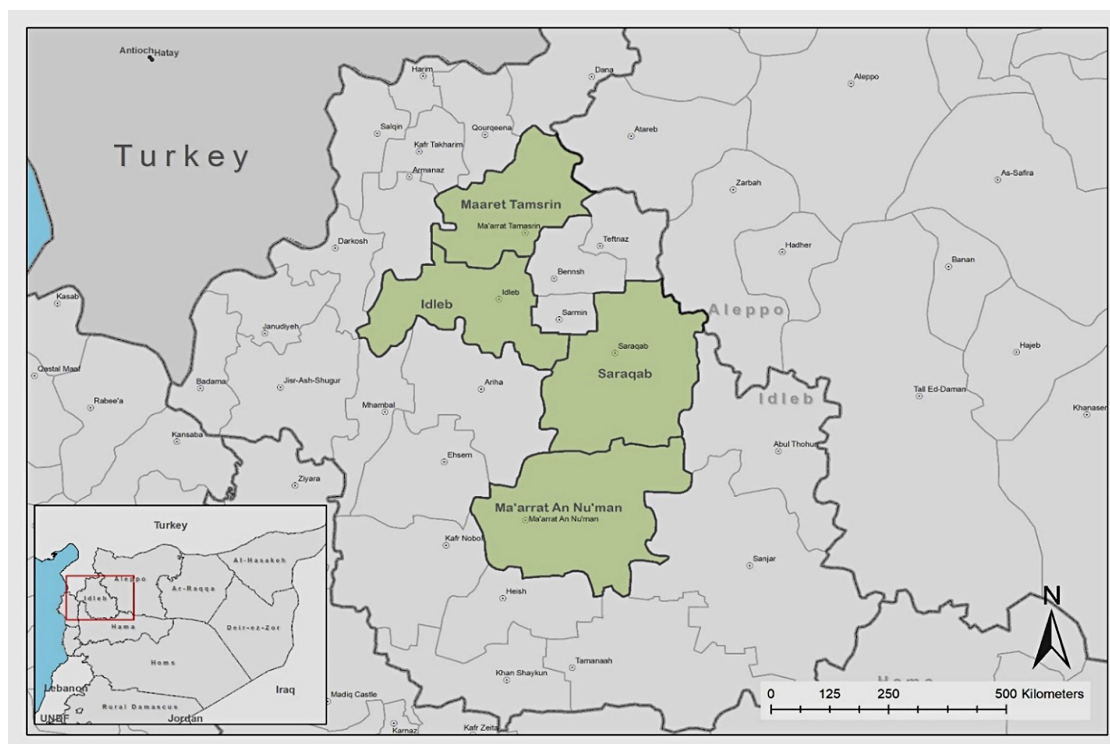


Figure 1. Study area. The inset map shows the research area in green, relative to the whole of Syria. Source: Humanitarian Data Exchange <https://data.humdata.org/dataset/syrian-arab-republic-administrative-boundaries-populated-places>

Table 1. Status of the cultivation of landraces by crop category across the study area (NW Syria) in 2019. PWC, previously widely cultivated, i.e. common and widely available in the communities in terms of yield and seed during the period 1999–2019; CWC, currently widely cultivated, i.e. previously widely cultivated, and still common and widely available.

Category	Common name	Scientific name	PWC	CWC
Cereals	Wheat	<i>Triticum aestivum</i>	10	5
	Broad bean	<i>Vicia faba</i>	4	3
	Chickpea	<i>Cicer arietinum</i>	6	2
Legumes	Common bean	<i>Phaseolus vulgaris</i>	5	3
	Cowpea	<i>Vigna unguiculata</i>	3	1
	Lentil	<i>Lens culinaris</i>	5	2
	Armenian cucumber	<i>Cucumis melo flexuosus</i>	5	1
	Eggplant	<i>Solanum melongena</i>	5	2
	Pepper	<i>Capsicum annum</i>	4	3
	Tomato	<i>Solanum lycopersicum</i>	4	1
Vegetables	Watermelon	<i>Citrullus lanatus</i>	3	2
	Zucchini	<i>Cucurbita pepo</i>	1	1
	Okra	<i>Abelmoschus esculentus</i>	3	2
	Squash	<i>Cucurbita pepo</i>	2	2
	Barley	<i>Hordeum vulgare</i>	4	1
Forages	Corn	<i>Zea mays</i>	5	2
Cash crops	Sunflower	<i>Helianthus annuus</i>	4	3
Totals			73	36

had been abandoned. Notably, none of the landraces that were previously widely cultivated are now only rarely cultivated or found only in restricted or very local cultivation. Furthermore, no new landraces were recorded and none of the landraces currently widely cultivated were previously only cultivated on a small scale. Figure 2 shows the decline in cultivation of the

landraces that were once widely grown by crop category, which all the interviewees agreed had been well adapted to the climatic conditions in their area.

Understanding why particular landraces were still in cultivation or not was an important part of this study. Interviewees and FG participants were asked to select one or more reasons from a list of possible causes

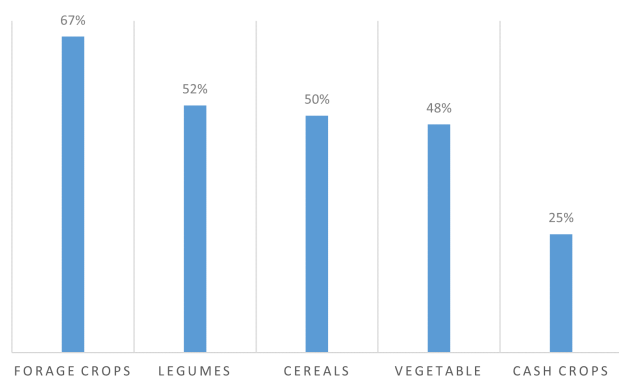


Figure 2. Percentage reduction in the number of landraces cultivated in the study area before and after 2019 by crop category.

developed by the study team for each crop category (Figure 3). Participants gave good taste, pest resistance and drought tolerance as the most important reasons for the continued use of crop landraces. Other factors, such as yield, historical interest and storage properties were deemed to be of less importance.

When offered a second set of reasons as to why they might continue cultivating these landraces, the most significant were local sale (cited by 41% of participants) and seed production (34%). Use for personal consumption (18%) and national sale (7%) were seen as less important.

Figure 4 shows that when 67 participating farmers were asked to consider why they no longer cultivated a particular landrace, the main factors given were lack of seeds (50%), competition from new hybrids (24%) and competition from more productive commercial varieties introduced from other parts of Syria (18%). Factors such as neglect by the competent authorities, disinterest amongst the next generation of farmers, lack of market demand and the absence of government control, were seen as less important.

When the farmers were asked about seed exchange, 90% confirmed the exchange of small quantities with other growers, with the remaining 10% open, in principle, to the practice of regular seed exchange. This was

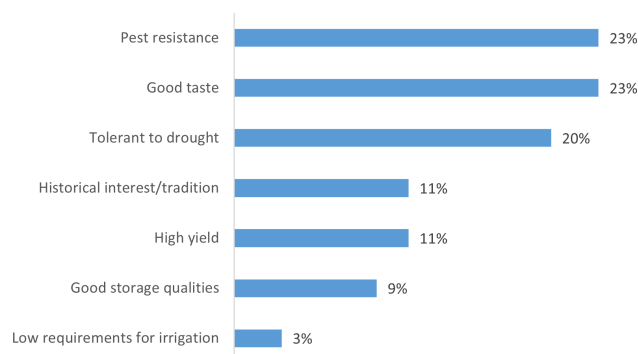


Figure 3. Reasons for the continued cultivation of crop landraces in NW Syria (based on the responses of the 75 interviewees and participants in the 25 FGs).

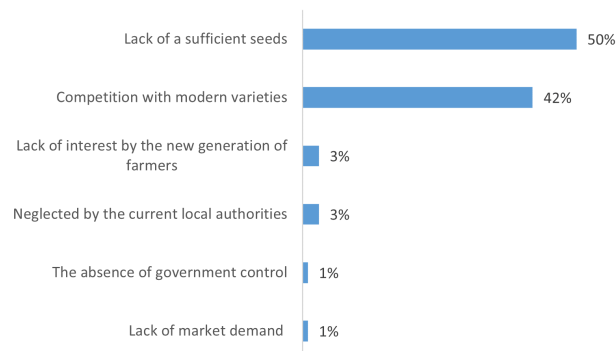


Figure 4. Reasons for farmers no longer cultivating crop landraces (based on the responses of 67 farmers).

reflected in their responses to questions on measures that might be taken to conserve landraces (Figure 5). All expressed an interest in conserving landraces, with the exchange of seeds with other farmers (61%) considered the most important protection measure, although conservation in genebanks was also seen to be important (22%). Although farmers did not consider the general conservation of crop landraces to be their responsibility, nevertheless, all those interviewed expressed a willingness to share their knowledge to support conservation of landraces through the establishment of a farmers' association.

Discussion

Prior to this initiative, the most recent available information on the status of crop landraces in Syria dated back to 2016 and focused on wheat. However, our 2019 study of vegetable, legume, cereal, forage, and cash-crop landraces reveals that about half of the landraces in cultivation in NW Syria 20 years ago are no longer cultivated. Although the range of crop species remains comparable, farmers no longer maintain or use all the landrace varieties that were cultivated in the past, indicating a significant loss of locally adapted crop diversity.

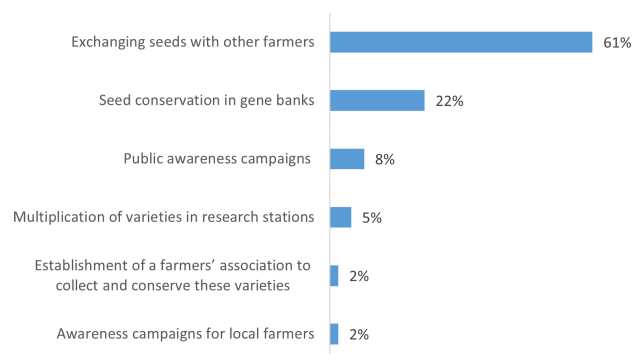


Figure 5. The importance given by the survey participants to different landrace conservation measures (based on the responses of the 75 interviewees and participants in the 25 FGs).

Although the study did not allow us to determine confidently whether this erosion of local genetic resources had increased since the start of the Syrian crisis in 2011, observations and knowledge gained throughout this period by the authors point strongly to an overall decline in biodiversity in NW Syria (both on-farm and in the wild), particularly due to social pressures such as migration and a dependency on collection of local natural resources. Although a number of the farmers who participated in the study did consider that an absence of government control and neglect by competent local authorities was a factor in the decline of their crop landraces, the ongoing crisis is clearly an underlying factor, pointing to the need for urgent interventions to protect these landraces from further genetic erosion. The reasons given for continuing to grow particular landraces were a mix of end-use and practical cultivation considerations. For example, flavour and texture were highlighted as important qualities, along with pest resistance and drought tolerance. The value of crop landraces for pest and disease resistance, as well as for drought tolerance, has been widely reported (Almekinders *et al*, 1994; FAO, 1995; Veteläinen *et al*, 2008; Dwivedi *et al*, 2016; FAO, 2019a)

Interestingly, yield was ranked lower than good taste, pest resistance and drought tolerance as a reason for continuing to cultivate crop landraces in this study, and equally with tradition and historical considerations. This indicates that while yield is often considered the most important aspect of crop production, landraces continue to be cultivated for other qualities that are not available in high-yielding varieties. The importance of local sales as a reason may also speak to the value of taste or tradition of the crop in the local area. One evident barrier to the continued cultivation of landraces is a lack of seeds (noted by 50% of the participating farmers), pointing to a need for landrace seed regeneration, which could be organized through the establishment of a farmers' cooperative. However, some landraces clearly yield sufficient seed quantities, as seed sales were given by 34% of the farmers as a reason for continuing to grow them. Notably, the availability of seeds of competing hybrid varieties, regardless of their suitability for cultivation in the local environment, was given by 24% of the farmers as a strong reason for their cultivation over and above landraces. The quantity of good-quality landrace seeds may also account for the fact that many landraces are abandoned, rather than continuing to grow them only on a very restricted scale.

Ninety percent of the participating farmers noted the occasional exchange of landrace seeds with other growers – an action reliant on local trust-based seed supply networks. This indicates continued interest in the cultivation of landraces and the need for a formal seed-multiplication and quality-control system to maintain their production following the cessation of the activities of the GOSM in NW Syria since the start of the crisis. The effectiveness of the efforts of the SIG to replace

that function in NW Syria has been limited and has not involved the seed multiplication of crop landraces.

Seed-exchange activities and local seed sales of landraces are a positive sign, although clearly insufficient to sustain landraces, given the study's finding that half of those in cultivation 20 years ago in the study area are no longer being grown by the farmers involved in the study, or based on the knowledge of other interviewed participants (agricultural researchers and engineers). These two ongoing activities may be the sole reason for the continued existence of the landraces that remain in cultivation, which might otherwise have become extinct. The extent of the remaining diversity could be determined through morphological and molecular genetic analyses.

Although the farmers involved in the study did not see themselves as the custodians of landraces, or believe that working together in farmers' associations would help, in the current crisis, they are nevertheless by default fulfilling this custodial role. The commonly held view was that this was the role of an external body, as had been the case prior to the crisis – a view illustrated by their willingness to support the practical measures initiated by the study team in the form of seed collection for *ex situ* storage and subsequent multiplication. Additional funds will be required to expand current landrace seed-multiplication activities and allow for their distribution amongst local farmers.

The results of this research point to the urgent need for complementary *in situ* (on-farm) and *ex situ* conservation to protect landraces that may otherwise be lost (Maxted *et al*, 1997; Camacho-Villa *et al*, 2005). Continuation of this work to create a local seedbank, and to support the regeneration and distribution of landrace seeds to farmers, could revitalize the cultivation and spread of these landraces in this war-torn region. The provision of seed samples to existing genebanks would provide additional protection against deleterious natural and human-induced events. The conservation of locally adapted crop landraces is an essential component of sustainable agricultural development for future food, nutrition and livelihood security for future generations in Syria and worldwide.

Acknowledgements

This research was supported by Cara (the Council for At-Risk Academics), United Kingdom. The Syrian research team wish to extend their thanks to Peter Hoebe, Richard Ellis, Ian Dawson and Meriel G. Jones for their assistance in developing an early draft of the manuscript.

Supplemental Data

[Interview questionnaire](#)

Author contributions

Munzer Al Darvish 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; Shelagh Kell provided academic guidance and support throughout the research process and contributed to the manuscript.

Conflict of interest statement

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

References

- ACU (2016). Syrian wheat reality. Report. 21. Assistance Coordination Unit. Şehitkamil/Gaziantep, Turkey
- Al-Ghazi, S. (2021). The wheat and bread crisis in Syria and its impact on the population. url: <https://orsam.org.tr/dhbanaliz/the-wheat-and-bread-crisis-in-syria-and-its-impact-on-the-population.pdf>.
- Alkiswany, H. (2012). Syrian conflict forces research institute to relocate. url: www.scidev.net/global/news/syrian-conflict-forces-research-institute-to-relocate/.
- Almekinders, C. J. M., Louwaars, N. P., and De Bruijn, G. H. (1994). Local seed systems and their importance for an improved seed supply in developing countries. *Euphytica* 78, 207–216. doi: <https://doi.org/10.1007/BF00027519>
- Camacho-Villa, T. C., Maxted, N., Scholten, M., and Ford-Lloyd, B. (2005). Defining and identifying crop landraces. *Plant Genetic Resources* 3(3), 373–384. doi: <https://doi.org/10.1079/PGR200591>
- Ceccarelli, S. and Grando, S. (2000). Barley landraces from the Fertile Crescent: a lesson for plant breeders. In *Genes in the Field: On-Farm Conservation of Crop Diversity*, ed. Brush, S. B. (IPGRI/IDRC/Lewis Publishers), 51–76.
- CRS (2015). Seed System Security Assessment: NW Syria. url: <https://seedsystem.org/wp-content/uploads/2016/05/SSSA-NW-Syria-Sept-2015-Report-for-distribution.pdf>.
- Damania, A. B. (1994). In situ conservation of biodiversity of wild progenitors of cereal crops in the Near East. *Biodiversity Letters* 2, 56–60. url: <https://www.jstor.org/stable/2999669>.
- Dwivedi, S. L., Ceccarelli, S., Blair, M. W., Upadhyaya, H. D., Are, A. K., and Ortiz, R. (2016). Landrace germplasm for improving yield and abiotic stress adaptation. *Trends in Plant Science* 21, 31–42. url: <https://www.sciencedirect.com/science/article/abs/pii/S1360138515002605>.
- FAO (1995). Syria: Country Report to the FAO International Technical Conference on Plant Genetic Resources. url: <https://www.fao.org/fileadmin/templates/agphome/documents/PGR/SoW1/east/SYRIANAR.PDF>.
- FAO (2003). Agro-ecological zones and farming systems. In *Fertilizer Use by Crop in the Syrian Arab Republic*. url: <http://www.fao.org/3/Y4732E/y4732e06.htm>
- FAO (2008). Climate Change and Biodiversity for Food and Agriculture. url: https://www.fao.org/uploads/media/FAO_2008a_climate_change_and_biodiversity_02.pdf.
- FAO (2011). Second Global Plan of Action for Plant Genetic Resources for Food and Agriculture. url: <https://www.fao.org/agriculture/crops/thematic-sitemap/theme/seeds-pgr/gpa/en/>.
- FAO (2019a). The State of the World's Biodiversity for Food and Agriculture. url: <http://www.fao.org/3/CA3129EN/CA3129EN.pdf>.
- FAO (2019b). Voluntary Guidelines for the Conservation and Sustainable Use of Farmers' Varieties/Landraces. url: <https://www.fao.org/family-farming/detail/en/c/1251445/>.
- Ficiciyan, A., Loos, J., Sievers-Glotzbach, S., and Tschardt, T. (2018). More than yield: ecosystem services of traditional versus modern crop varieties revisited. *Sustainability* 10(8), 2834–2834. doi: <https://doi.org/10.3390/su10082834>
- Frison, C., López, F., and Esquinas-Alcázar, J. T. (2011). Plant Genetic Resources and Food Security: Stakeholder Perspectives on the International Treaty on Plant Genetic Resources for Food and Agriculture (New York, NY). doi: <https://www.fao.org/3/bb143e/bb143e.pdf>
- GenRes Bridge Project Consortium, ECPGR, ERFP and EUFORGEN (2021). Genetic Resources Strategy for Europe. url: <http://www.genresbridge.eu/fileadmin/templates/Genres/Uploads/Documents/GRS4E.pdf>.
- Gewin, V. (2015). But who's preserving them? As climate change makes crop diversity even more important, gene banks struggle to stay afloat. url: <https://ensia.com/features/crop-gene-banks-preserved-the-future-of-agriculture-but-whos-preserving-them>.
- GOSM (2017). The project of restoring local Syrian wheat varieties and preventing their loss and deterioration and working to multiply them. url: <https://gosm-sy.org/the-project-of-restoring-local-syrian-wheat-varieties-and-preventing-their-loss-and-deterioration-and-working-to-multiply-them/>.
- ICARDA (2017). Strategic Plan 2017–2026. url: <https://www.icarda.org/publications/11367/icarda-strategic-plan-2017-2026>.
- Jaradat, A. A. (2017). Agriculture in the Fertile Crescent: continuity and change under climate change. *CAB Reviews* 12(34), 1–33. doi: <https://doi.org/10.1079/PAVSNNR201712034>
- Kelley, C. P., Mohtadi, S., Cane, M. A., Seager, R., and Kushnir, Y. (2015). Climate change in the

- Fertile Crescent and implications of the recent Syrian drought. *Proceedings of the National Academy of Sciences* 112(11), 3241–3246. doi: <https://doi.org/10.1073/pnas.1421533112>
- Maxted, N., Ford-Lloyd, B., and Hawkes, J. G. (1997). Complementary conservation strategies. In *Plant Genetic Conservation: The In Situ Approach.*, ed. Maxted, N., Ford-Lloyd, B., and Hawkes, J. G. (Chapman and Hall), 15-39.
- Maxted, N., Kell, S., and Brehm, J. M. (2011). Options to Promote Food Security: On-Farm Management and In Situ Conservation of Plant Genetic Resources for Food and Agriculture. Background Study Paper No. 51. url: <https://www.fao.org/3/am489e/am489e.pdf>.
- Mazid, A., Shideed, K., and Amri, A. (2014). Assessment of on-farm conservation of dryland agrobiodiversity and its impact on rural livelihoods in the Fertile Crescent. *Renewable Agriculture and Food Systems* 29(4), 366–377. doi: <http://dx.doi.org/10.1017/S1742170513000240>
- Mohammed, S. A., Alkerdi, A., Nagy, J., and Harsányi, E. (2019). Syrian crisis repercussions on the agricultural sector: Case study of wheat, cotton and olives. *Regional Science Policy and Practice* 12(3), 519–537. doi: <https://doi.org/10.1111/rsp3.12222>
- MSEA (2009). The fourth national report on biodiversity in the Syrian Arab Republic. url: <https://www.cbd.int/doc/world/sy/sy-nr-04-en.pdf>.
- Petropoulos, S. A., Barros, L., and Ferreira, I. C. F. R. (2019). Editorial: Rediscovering local landraces: Shaping horticulture for the future. *Frontiers in Plant Science* 12. doi: <https://doi.org/10.3389/fpls.2019.00126>
- Van De Wouw, M., Kik, C., Van Hintum, T., Van Treuren, R., and Visser, B. (2010). Genetic erosion in crops: Concept, research results and challenges. *Plant Genetic Resources* 8(1), 1–15. doi: <https://doi.org/10.1017/S1479262109990062>
- Vavilov, N. I. (1926). *Studies in the Origin of Cultivated Plants* (Leningrad: Institute of Applied Botany and Plant Breeding).
- Veteläinen, M., Negri, V., and Maxted, N. (2008). European landraces: on-farm conservation, management and use. *Biodiversity Technical Bulletin No. 15*. url: <https://hdl.handle.net/10568/106154>.
- Willcox, G. (2012). The beginnings of cereal cultivation and domestication in Southwest Asia. In *A Companion to the Archaeology of the Ancient Near East*, ed. Potts, D. (Blackwell, Chichester), 163-180.



Optimum contribution selection (OCS) analyses prompted successful conservation actions for Faroese horse population

Anne Kettunen ^{*,a,b}, Signa Kallsøy Joensen ^c and Peer Berg ^{a,d}

^a The Nordic Genetic Resource Center (NordGen), c/o Norwegian University of Biosciences, P.O. Box 5003, NMBU, NO-1432 Ås, Norway

^b Nofima AS, P.O. Box 210, NO-1431 Ås, Norway

^c Felagið Føroysk Ross, á Signabø 64, 416 Signabøur, Faroe Islands

^d Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Ås, Norway

Abstract: The Faroese horse, an endangered indigenous horse breed, is a part of the cultural and societal heritage of the Faroe Islands. Population history describes a severe bottleneck, prompting for quantification of the genetic diversity (level of inbreeding, probability of gene origin, effective population size) and assessment of sustainable conservation potential (Optimum Contribution Selection, OCS) of the Faroese horse population. The pedigree completeness (PCI) of the Faroese horse is adequate for a realistic estimation of the level of inbreeding ($PCI_5 = 0.96$). In concordance with the known population history, the average inbreeding is exceptionally high; in the last cohort, it was equal to 26.8%. An estimate of the effective population size, based on individual increase in inbreeding and coancestry, accounting for the whole population history, was eight. OCS offers a tool to understand and control the increase in the average relationships in the population. Within a fixed number of matings, the repetitive use of stallions resulted in the lowest level of average relationships. Successful follow-up of mating schemes planned together with a holistic assessment of the suitability of an individual as a breeding candidate, will minimize the increase in inbreeding in future generations and maximize the possibility to increase the census size of the Faroese horse population.

Keywords: conservation, native breed, horse, effective population size, optimum contribution selection

Citation: Kettunen, A., Joensen, S. K., Berg, P. (2022). Optimum contribution selection (OCS) analyses prompted successful conservation actions for Faroese horse population. *Genetic Resources* 3 (5), 59–67. doi: [10.46265/genresj.KKXV5870](https://doi.org/10.46265/genresj.KKXV5870).

© Copyright 2022 the Authors.

This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

The Faroese horse (*Føroyska rossið* in Faroese) represents an important part of the agricultural history and cultural integrity of the Faroe Islands. According to the Domestic Animal Diversity Information System (DAD-IS) of the Food and Agriculture Organization of the UN (FAO), the breed status is defined as critical and at risk. The history of the Faroese horse is not well documented and there is ambiguous evi-

dence of the origin of the Faroese horse. Most likely the Faroese horse descended from Scandinavian and/or Celtic horse breeds brought to the Faroe Islands by settlers in 500–800 CE (Bjørk, 1984; Joensen, 2019). In written sources, the Faroese horse and its use are first described in the 1600s (Bjørk, 1984; Joensen, 2019). Despite their small size, the Faroese horses were traditionally used for carrying heavy loads, only seldom used for riding, and never as draught horses as there were no suitable roads for that (Bjørk, 1984). Horses were only brought to the villages when they were needed as a working force to carry manure to the fields, peat to the houses or during the harvest of hay and grain (Bjørk,

*Corresponding author: Anne Kettunen
(<mailto:anne.kettunen@nofima.no>)

1984). Outside of these periods of gruelling work, horses were kept free ranging without supplemental feed all year round. Consequently, the Faroese horse developed into a small (115–125cm at withers), strong, resilient horse well adapted to the demanding climate, terrain and vegetation of the Faroe Islands. At its highest, the population size of Faroese horses has been approximately 800 individuals (Bjørk, 1984).

In the late 19th century, a shift of labour from agriculture to fisheries and the mechanization of agriculture decreased the importance of horses as working power for agriculture. At the same time, the Faroese horse became a valuable export commodity; a large number of Faroese horses were exported to the British Isles as pit ponies for the coal mining industry (Bjørk, 1984; Joensen, 2019). The need for pit ponies was increased when the use of women and children as workforce in the mines was made illegal. The reduced population size and decreased interest in pure Faroese horses set off demand for the importation of foreign breeds to the Faroe Islands. Consequently, Norwegian Fjord horses and Icelandic horses were imported and interbred with Faroese horses. Crossbreeding increased the height at withers (125–132cm) and improved the working ability of the horse, but had a deteriorating effect on the overwintering ability (Bjørk, 1984). Although the documentation of the population management is deficient, heavy restrictions on stallion use during the 1930s when foreign breeds were strongly favoured, are described in literature (Bjørk, 1984). By the 1960s, the Faroese horse population was close to extinction with only five individuals alive.

Currently, a small population of Faroese horses is maintained on the Faroe Islands by hobby breeders. Today, this unique breed is used for recreational purposes and tourism. The current population stems from a few horses born between the 1940s and 1960s. In 1978, the organization *Felagið Føroysk Ross* was established to bring together people dedicated to conserving this native breed. The genetic variation in the Faroese horse population using 12 microsatellite markers was earlier assessed by Mikko et al (2004). As expected, the population had low genetic variation but did not show any apparent signs of inbreeding. In recent years, the interest in the sustainable conservation of Faroese horses has greatly increased. Given the traditional knowledge of the population history, and due to the limited success in increasing the population size, there is a need for a knowledge-based management plan for this unique horse breed.

Genetic diversity describes the range of different traits inherited in a population. Genetic diversity is a prerequisite for selective breeding and critical for a population's adaptation potential in changing environments. Endangered local breeds typically have small census sizes and low genetic diversity. Consequently, minimization of the rate of inbreeding is imperative for the sustainable conservation of these breeds. Optimum Contribution Selection (OCS) is a method that enables maximiza-

tion of the selected parents' genetic level and simultaneously constrains their average coancestry at a desired level (Meuwissen, 1997; Henryon et al, 2015). OCS is used in many conservation programmes, and for example, suggested by Nielsen and Kargo (2020) as part of the conservation strategy to save the endangered Jutland horse.

Our hypothesis was that the Faroese horse expresses extremely low genetic diversity and high levels of inbreeding, and that further rapid increase in inbreeding can be circumvented by using OCS. To test this hypothesis, we conducted a pedigree analysis of the Faroese horse population to assess the level of genetic diversity. Effective population size (N_e), the key parameter for describing genetic diversity in animal populations, was estimated with several methods. Additionally, we used OCS to investigate the possibilities for sustainable management of this horse breed. Based on these results, management recommendations were drafted.

Materials and methods

Data

Pedigree data, comprising horses born between 1917 and 2016, were obtained from the *Felagið Føroysk Ross*. Out of the 178 individuals, 87 were males and 91 females.

In the 1960s the population of Faroese horses experienced a severe bottleneck with only five individuals alive (Figure 1). These were one stallion born in 1962 and four mares born in 1946, 1952, 1956 and 1958. One of the mares is the dam of two of the other dams, both being a result of a sire–daughter mating. One of the mares alive in the 1960s is unrelated to the other four individuals. The number of horses born per year is presented in Figure 2. A very low number of foals were born yearly until the recent years when invigorated national efforts for the sustainable conservation of the Faroese horse have resulted in increased population size (Figure 2). Between 2000 and 2016, a total of 92 foals were born, out of which 66 were alive at the time of the study (December 2016). It is worth mentioning that additional 25 foals have been born between 2017 and August 2019.

Statistical methods

Population analyses

Pedigree completeness, level of inbreeding and additive genetic relationships were calculated using EVA 2.1 (Berg et al, 2006; Henryon et al, 2015). Two birth years were combined into biannual cohorts from the year 1991 and onwards due to the low number of foals born per year. Estimates of effective population size and parameters derived from the probabilities of gene origin were obtained by ENDOG v.4.8 (Gutiérrez and Goyache, 2005). More specifically, different methods were used to derive ΔF and thereafter effective population was com-

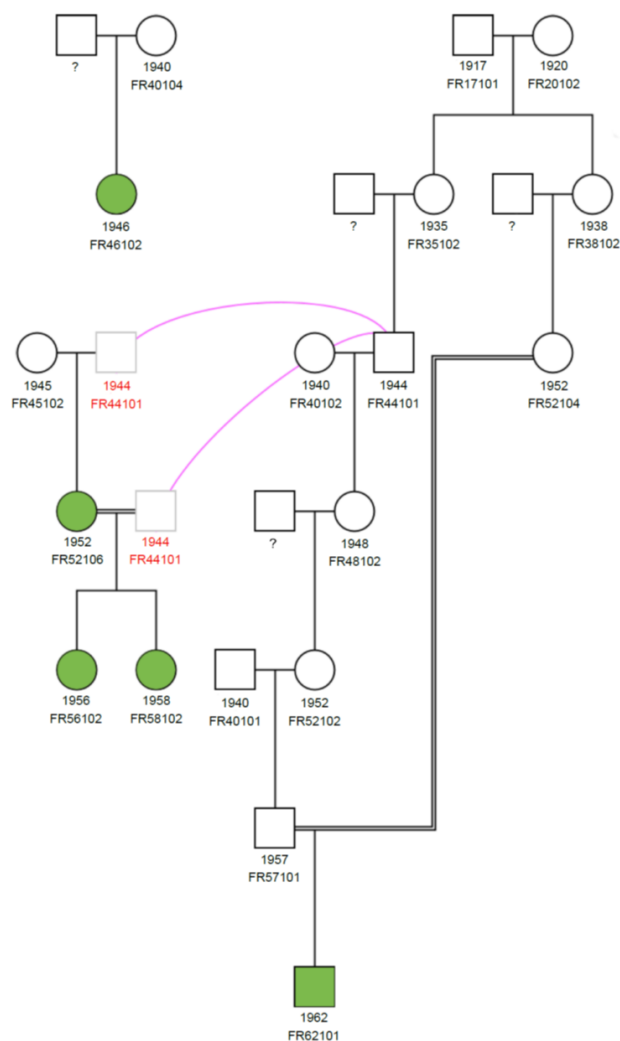


Figure 1. Pedigree for animals in the bottleneck. Stallions are marked as squares and mares as circles. Symbols with green filling are the five individuals alive in the 1960s.

puted as $N_e = \frac{1}{2\Delta F}$, using the same estimators of rate of inbreeding as in a previous study on Norwegian Lundehund (Kettunen *et al*, 2017).

In short, three regression coefficients of the individual inbreeding coefficients on the number (method identification in parentheses) of i) complete generations (Ia), ii) maximum number of generations (Ib) and ii) equivalent generations (Ic) traced back to founders for each individual, were computed to give information on the lower, upper and 'real' limits of $N_e = \frac{1}{2b}$. Further, two regression methods were used to approximate ΔF ; first, the regression coefficient of the individual inbreeding coefficients on birth years (II), and second the regression coefficient of the inbreeding coefficients regressed on the number of equivalent generations (IV). The average generation interval (Gutiérrez *et al*, 2003) was used to define the increase in inbreeding between two generations. In two other methods values of $\log(1 - F_i)$ were regressed either on birth years (IIIa) or complete generation equivalents (IIIb) (Pérez-Enciso, 1995). Additionally, individual increases in inbreeding (V) (Gutiérrez

et al, 2008, 2009) and increase in coancestry (VI) for all pairs of individuals in the reference population (Cervantes *et al*, 2011) were used for estimation of N_e and the standard error of the estimated N_e . Additional details of the methods used are presented in Kettunen *et al* (2017) and references therein.

Parameters derived from the probabilities of gene origin, that is the effective number of founders (f_e), ancestors (f_a), non-founders (f_{ne}) and the founder genome equivalent (f_{ge}) were used to describe the genetic variability in the Faroese horse population. The definition and calculation of these parameters can be found in literature (Lacy, 1989, 1995; Boichard *et al*, 1997; Caballero and Toro, 2000). These parameters were used to calculate the degree of genetic diversity relative to the base accounting for loss of diversity due to unequal founder contribution, bottlenecks and genetic drift (Kettunen *et al*, 2017). Parameters f_e , f_a and f_{ne} were obtained from ENDOG whereas f_{ge} was calculated as $\frac{1}{2\bar{\alpha}}$, where $\bar{\alpha}$ was the average coancestry of all living individuals (Caballero and Toro, 2000).

Optimum contribution selection

EVA 2.1 software was used for the OCS analyses (Berg *et al*, 2006; Henryon *et al*, 2015). Horses born between 1995 and 2014 were considered breeding candidates. Individuals with known health or reproductive problems were excluded as breeding candidates prior to OCS analyses. This resulted in a total of 24 males and 28 females being available for breeding. Genetic contributions were optimized for 6 to 12 matings, constraining the number of matings allowed for each stallion from 1 to 4. Genetic contributions were optimized using default algorithm parameters (Supplemental data) with full penalty on average relationships; this equals zero weight on genetic merit with all focus on minimization of the average relationships.

Results and discussion

Pedigree completeness and inbreeding

Pedigree completeness (MacCluer *et al*, 1983) was computed three (PCI3), five (PCI5) and seven (PCI7) generations back (Figure 3). For the foals born in 2016, PCI3, PCI5 and PCI7 were 1.0, 0.96 and 0.83, respectively. PCI is essentially a measure of the proportion of known ancestral contributions that could contribute to inbreeding. Consequently, low population level PCI will result in underestimation of the level of inbreeding and overestimation of the level of genetic diversity, as unknown relationships are treated as unrelatedness. Giontella *et al* (2019) reported PCI of 90% and 70% for the third and fifth parental generation of the indigenous Maremmano horse breed, respectively. PCIs for three different reference populations of Hokkaido native horses were reported in Onogi *et al* (2017). Estimates for the last reference population (2006–2015) were identical to those for Faroese horses.

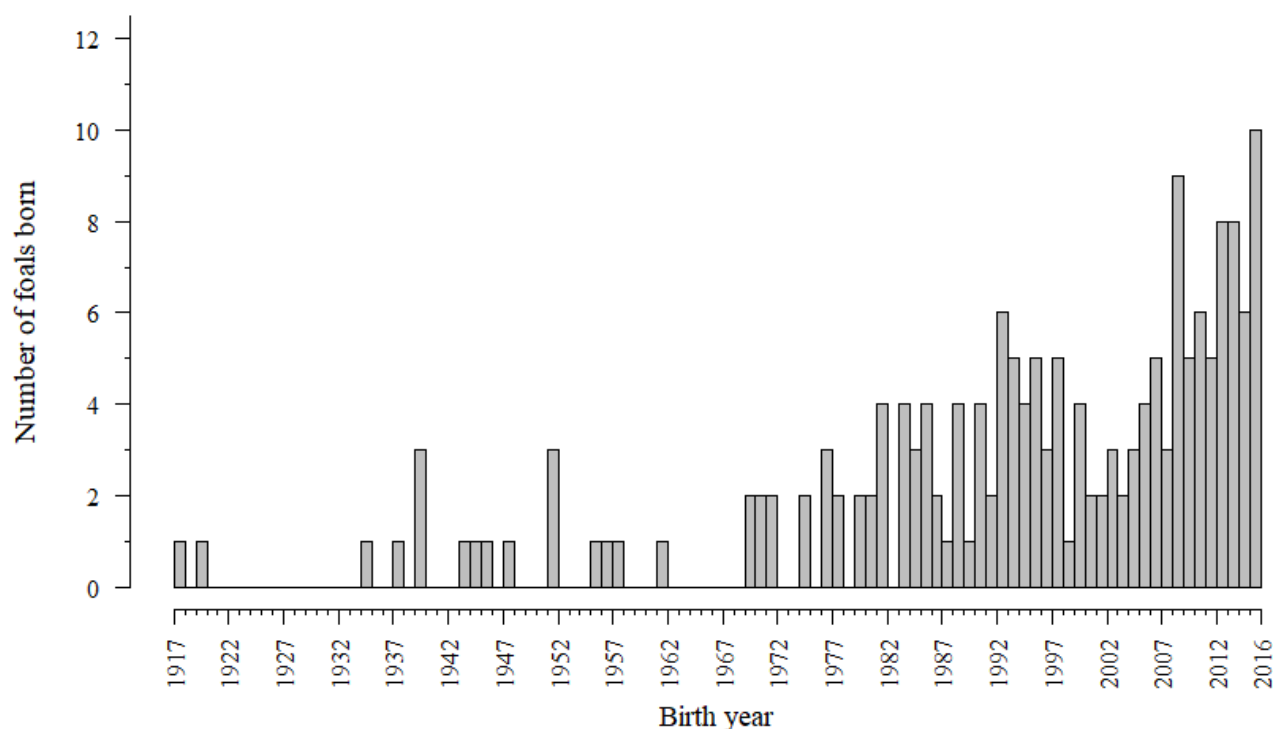


Figure 2. Number of individuals per birth year between 1917–2016.

The pedigree completeness of the tiny pedigree of the Faroese horse is adequate for a realistic estimation of the level of inbreeding.

The average co-ancestry and inbreeding in the last cohort in this study (foals born in 2016) were 32.7% and 26.8%, respectively. As the number of breeding animals was very limited, the use of very old individuals for breeding was common. For individuals born in 1980 and onwards the average age of dams was 11.7 years

and sires 9.8 years, resulting in an average generation interval of 10.7 years. A total of 22 matings in the whole pedigree were between half-sibs (12.4%), 3 between full-sibs (1.7%) and 15 (8.4%) between parent and offspring. The average inbreeding over birth years fluctuated, and inbreeding in some birth years was governed by one or very few individuals (Figure 4). The individual inbreeding coefficients in the total pedigree (excluding founders and half-founders) varied between 0.8 and 41.3%.

Sire–daughter mating between the same two individuals resulted in highly inbred individuals born in 1956 and 1958. There was a rapid increase in the level of inbreeding during the 1970s. This was a consequence of the bottleneck in the 1960s, when only five individuals were alive. From 1980 onwards, the increase in inbreeding was moderate (Figure 4), with exception of the year 1990 (2004), where only one (two) foal(s) was born with a very high inbreeding coefficient. The overall average inbreeding was 21.9%, with 89.9% inbred animals. A total of 48.2% individuals have inbreeding coefficient corresponding to half-sib to full-sib mating, and 11.3% of the horses had inbreeding coefficients above 30%. Average inbreeding of the Faroese horse is much higher than that reported for different horse breeds in literature; average inbreeding in native horse populations is typically below 10% (Gandini et al, 1992; Sairanen et al, 2009; Duru, 2017; Onogi et al, 2017; Giontella et al, 2019; Mancin et al,

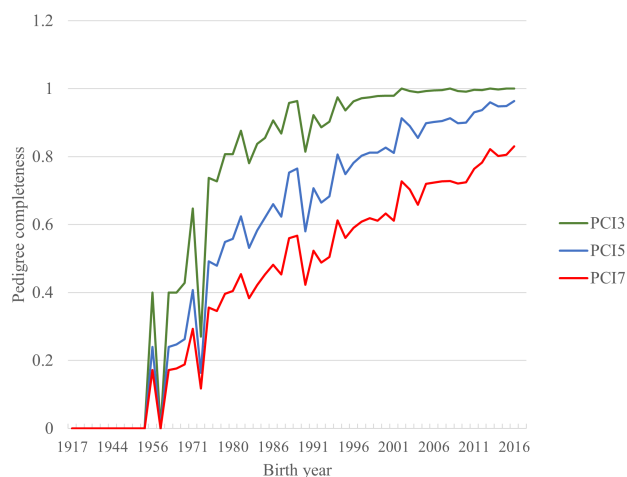


Figure 3. Pedigree completeness three (PCI3), five (PCI5) and seven (PCI7) generations back over birth years.

2020; Perdomo-González *et al*, 2020; Poyato-Bonilla *et al*, 2020). Slightly higher pedigree-based inbreeding coefficients have been reported for Old Kladruher horse, Warmblood trotter and Gotland Russ (Vostrá-Vydrová *et al*, 2016; Kvist *et al*, 2019). Similarly to the Faroese horse population, extremely high inbreeding (32.5%) has been reported in a small population of the endangered Sorraia horse (Luís *et al*, 2007).

Average coancestry among parents of a cohort expresses the expected level of inbreeding of the progeny that results from random mating. The realized inbreeding of the progeny in Faroese horses is slightly lower than the expected inbreeding. This indicates that, despite the high relatedness between available breeding candidates, breeders have mated individuals less related than expected with random mating (Figure 4). This said, the same phenomenon could arise if matings resulting in highly inbred progeny are partly unsuccessful, e.g. due to lethal allele combinations, and consequently fewer offspring would be born from these matings.

Although no apparent signs of inbreeding in the Faroese horse were found in Mikko *et al* (2004), it is expected that matings resulting in extremely high inbreeding are not always successful. Adverse effects of inbreeding on reproduction have been documented in literature. Results from Sairanen *et al* (2009) confirmed that intense inbreeding as well as the age of both mare and stallion, have an adverse effect on foaling rate. Reproductive problems, such as retained placenta and reduced sperm motility have been reported in Friesian horses and Shetland ponies with inbreeding above 7% and 2%, respectively (Onogi *et al* (2017) and references therein). Although the population size of Faroese horses has increased during the past three decades, some mares had repeated fertility problems (Joensen, 2019). These mares repeatedly experienced miscarriages, and repeatedly remained empty despite successful mating. A recent study on the Faroese horse concluded that 30% of the mares are infected by equine endometritis, yet infection status was not fully coherent with the fertility status of the mares (Joensen, 2019). The magnitude of inbreeding depression on the Faroese horse reproduction has not been documented. That said, given the generally high level of relatedness of the population, mating of highly related individuals should be avoided.

Genetic contributions of founders and ancestors

The base population, defined as the individuals with one or more unknown parents, consisted of ten individuals. The actual base population, where an animal with one unknown parent was defined as a half-founder, consisted of eight animals. The contribution of the ten founder individuals to the last recorded cohort of 2016 varied between 3.0–12.3%. The highest genetic contribution of an ancestor to the most recent cohort was 48.5% from the only living stallion in the 1960s bottleneck (Figure 1). The second-largest genetic contribution of 37.1% was from its son born in 1972. Only one

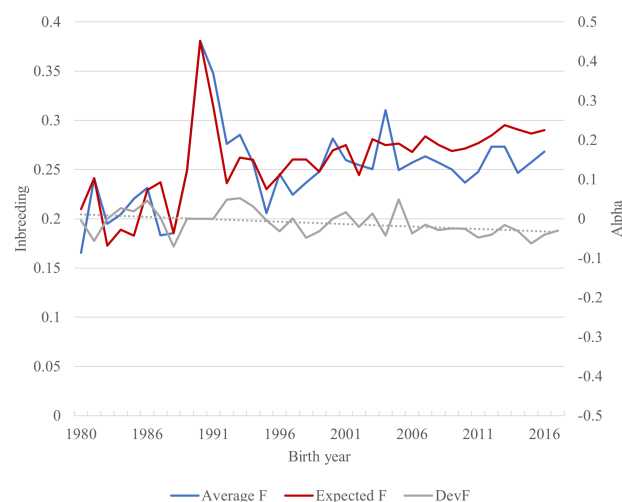


Figure 4. Average observed and expected inbreeding and deviation from random mating (DevF) between birth years 1980 and 2016.

of the mares alive during the 1960s was unrelated, and contributed 24.6% to the 2016 cohort. The three remaining mares alive during the bottleneck were sired by one sire only; its genetic contribution to the latest cohort was 22.3%. The genetic contributions of the dam and its two daughters were 15.2%, 16.3% and 7.0%, respectively. Only two ancestral individuals accounted for 50% of the variation in the population with known parents. The genetic contribution of the ancestors sums up to more than 100% as the genetic contributions in this extremely small pedigree are highly dependent.

Genetic diversity and effective population size

Effective number of founders (f_e), ancestors (f_a), non-founders (f_{ne}) and genomic equivalents (f_{ge}) were 9, 3, 1.7 and 1.4, respectively, with reference population of all animals with both parents known. Parameter f_e is defined as the number of equally contributing founders that would result in the genetic diversity of the population under study. It only accounts for the loss in genetic variability due to unequal contribution of founders (Lacy, 1989), thus it is not very useful for assessing genetic diversity. Parameter f_a is defined as the minimum number of ancestors explaining the complete genetic diversity of the population, and accounts for potential bottlenecks in the pedigree (Boichard *et al*, 1997). The amount of genetic drift since the foundation of the population is reflected by f_{ne} . Finally, f_{ge} represents the cumulative loss of genetic diversity since the base population and directly relates to N_e (Lacy, 1989, 1995; Caballero and Toro, 2000).

We used probability of gene origin parameters to derive genetic diversity measures to estimate the degree of genetic diversity relative to the base accounting for loss of diversity due to unequal founder contribution, bottlenecks and genetic drift. The total loss of genetic diversity relative to the base population was 35.8%

($GD = 1 - \frac{1}{2f_{ge}} = 0.642$) (Lacy, 1995; Caballero and Toro, 2000). Proportionally, 84.5% of this loss could be allocated to bottlenecks and random genetic drift ($1 - \frac{f_{ge}}{f_e}$). Consequently, 5.6% of the genetic diversity relative to the base ($GD^* = 1 - \frac{1}{2f_e} = 0.944$) was lost due to unequal founder contribution (15.5% of the total diversity loss) (Lacy, 1995).

In literature, estimates of parameters of gene origin are frequently used to describe the genetic diversity of horse populations (Duru, 2017; Onogi et al, 2017; Giontella et al, 2019; Mancin et al, 2020; Perdomo-González et al, 2020). Ratios of the parameters have been used to interpret the loss of genetic diversity (Onogi et al, 2017; Perdomo-González et al, 2020), but ignoring the definitions of the quantification of the proportion lost/retained relative to the base (GD, GD^* , GD^*-GD) in a quantitative manner (Lacy, 1995). This leads to ambiguous interpretations of the parameter ratios as indicators of the origin of the loss of the genetic variation between studies. Following Lacy (1995), the complete loss of genetic variation relative to the base for Pura Raza Español (Perdomo-González et al, 2020), Turkish Arab horse (Duru, 2017), the Italian Maremmano horse (Giontella et al, 2019), the Hokkaido native horse (Onogi et al, 2017) and Italian heavy draught horse (Mancin et al, 2020) is small compared to the Faroese horse: 5.7%, 5.2%, 3.6%, 2.7% and 0.7%, respectively. A slightly higher degree of loss in genetic diversity was estimated for the endangered Old Kladruber horse: 10.5–17%, depending on the reference population (Vostrá-Vydrová et al, 2016). The genetic diversity of the Faroese horse based on the pedigree analysis is extremely low.

Regression coefficients of the individual inbreeding coefficients on the number of i) complete generations (Ia), ii) maximum number of generations (Ib) and ii) equivalent generations (Ic) traced back to founders for each individual were computed. Method Ia and Ic give information on the lower and upper limits of N_e , whereas method Ib should indicate the 'real' N_e (Gutiérrez and Goyache, 2005). The estimates of N_e from the regression-based methods ranged from 8.54 to 24.39 (Table 1). Methods V and VI (Table 1), based on individual increase in inbreeding and coancestry, account for the whole population history estimated N_e to be 8. Estimates of N_e for local breeds such as Turkish Arab, Italian Maremmano, Hungarian Hucul and Italian heavy draught horse estimated with methods V and VI (Table 1) have been considerably larger than those for the Faroese horse: 42–97 (Duru, 2017; Somogyvári et al, 2018; Giontella et al, 2019; Mancin et al, 2020). Despite the slight decrease in the rate of inbreeding in recent years, all the estimates of N_e for the Faroese horse are critically low, and far from the recommended N_e of 50 to 100 to ensure the sustainable management of this endangered horse breed.

Optimum contribution selection

To test whether optimum contribution selection is successful in alleviating problems with inbreeding, we assessed breeding scenarios of 6 to 12 matings, where males were mated with a maximum of 1 to 4 females, relative to the average relationship (Figure 5) and selected individuals (Figure 6).

Within a fixed number of matings the repetitive use of stallions resulted in the lowest level of average relationships, although the differences between scenarios were very small and at maximum approximately 0.4% unit (Figure 5, maxmate = 1, 6 vs 12 matings). In an extremely small and inbred population, as is the case of the Faroese horse, an increase in the number of selected individuals inevitably results in the selection of more related individuals.

A minimum of four and a maximum of seven stallions were selected for breeding over all the scenarios allowing repetitive use of stallions. A subset of the same three stallions was selected in all scenarios, and in total only seven out of the 24 available stallions were ever selected across all scenarios (Figure 6). The additive genetic relationship of each of the selected males with all candidate males (0.60–0.62) and females (0.53–0.59) was equal to or lower than the average amongst male candidates (0.62) or between male and female candidates (0.59). Additionally, the additive genetic relationship of the selected males with the individuals born during the last two years (2015–2016) was lower than the average of all candidate males: 0.57 vs. 0.60. Similarly, the additive genetic relationship of the selected females with all candidate females (0.60) and males (0.57) was lower than that amongst female candidates (0.62) or between female and male candidates (0.59). The additive genetic relationship of the selected females with individuals in the last two

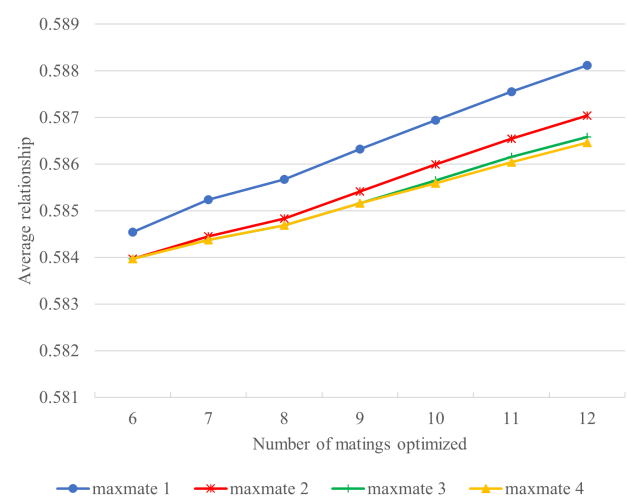


Figure 5. Average relationship of selected individuals in different breeding scenarios. Number of matings per stallion was constrained to 1–4 (maxmate) and a total of 6–12 matings were optimized. The points represent the lowest possible average relationship for a given number of matings and constraints of stallion use.

cohorts was lower than that of all female candidates: 0.58 vs. 0.60.

The potential of OCS for optimization of genetic gain in performance and increase in relatedness in e.g. the Franches-Montagnes horse, Menorca horse and Norwegian and North-Swedish trotter has been assessed by Hasler *et al* (2011), Olsen *et al* (2013) and Solé *et al* (2013). Similar to the Faroese horse, full weight on average relationships and minimization of inbreeding when optimizing matings for the endangered Jutland

horse were used in Nielsen and Kargo (2020); authors reported that preselection of sires and OCS successfully lowered the average inbreeding compared to random mating. Sustainable breeding of an extremely small and a priori highly inbred population is of great importance. Successful follow-up of mating schemes planned with OCS helps to avoid a rapid increase in inbreeding in future generations, as well as minimizes inbreeding of single matings. Therefore, whether an individual is eligible as a breeding candidate should be evaluated

Table 1. Estimates of effective population size (N_e) calculated with different methods. For methods V and VI, standard error in parentheses. *, Reference population of individuals with both parents known ($N = 168$); **, $N = 163$. Full description of methods II, IIIb, V and VI are found in Gutiérrez *et al* (2003), Pérez-Enciso (1995), Gutiérrez *et al* (2008), Gutiérrez *et al* (2009) and Cervantes *et al* (2011), respectively.

Method		N_e
Whole pedigree		
Ia:	Regression on complete generations	8.54
Ib:	Regression on maximum generations	24.39
Ic:	Regression on equivalent generations	12.69
II:	Regression on birth date	11.04
IIIa:	Log regression on birth date	11.57
Restricted pedigree*		
IIIb:	Log regression on equivalent generations	15.37
IV**:	Regression on equivalent generations	14.16
V:	Individual increase in inbreeding	7.99 (1.35)
VI:	Individual increase in co-ancestry	7.88 (0.43)

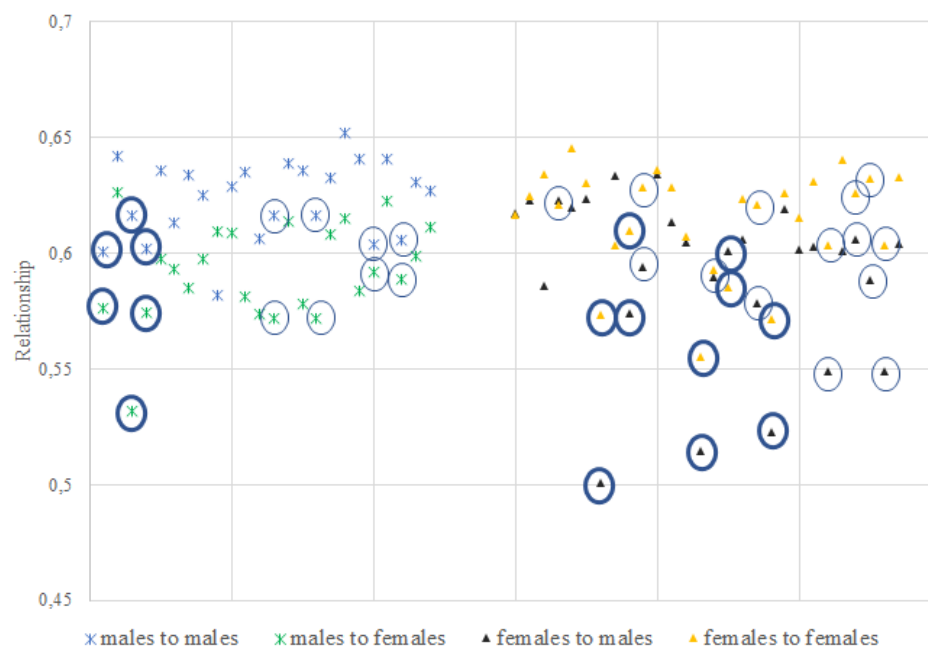


Figure 6. Breeding candidates with their relationships to all candidate males ($N = 24$) and females ($N = 28$) (each candidate having two indicators, one for same sex and one for opposite sex). Individuals selected in any of the scenarios are marked with circles and individuals selected for all scenarios with 6–12 requested matings and repetitive use of males (2–4) with bold circles.

thoroughly relative to the health of the individual and possible previous experiences regarding fertility and mothering characters. This is to avoid that the planned mating will not be realized or does not result in viable progeny. The *Felagið Føroysk Ross* has adopted the use of a database, *Føroya Fongur*, to collect all information (e.g. pedigree, health, mating, progeny) on the Faroese horse population. Information collected in the database on reproduction and health will assist in the recognition of possible inbreeding depression, but this process is still in its infancy. The Government of the Faroe Islands provides support (currently managed by the Agricultural Agency of the Faroe Islands) to horse owners if their horses are registered in the database and are producing offspring. Unfortunately, the breeder can receive support even if the planned mating would not be appropriate relative to the resulting inbreeding of the offspring. Overall, the maximization of reproductive success is of high priority to be able to expand the census size.

Conclusions and recommendations

Results from this study are in accordance with the documented population history of the Faroese horse; the population has extremely high average relatedness and inbreeding, and low effective population size. Consequently, the census size should be expanded as it sets the limit of constraining the future rate of inbreeding. It is recommended to optimize the contributions of parents to the next cohort to minimize increases in average coancestry of the population. Due to the expected inbreeding depression, the fitness-related traits should be recorded in the database and carefully monitored. To increase the interest in the breed, actions to improve the economic value of the Faroese horse, e.g. through export and increased use in tourism should be promoted. Molecular genetic characterization is recommended to be able to assess the additive genetic relationships between individuals accurately and to heighten the potential of OCS in conserving the Faroese horse.

Acknowledgements

We thank the *Felagið Føroysk Ross* for providing the data.

Supplemental data

[Algorithm parameters used for EVA analyses](#)

Author contributions

Signa Kallsoy Joensen compiled and quality checked the pedigree data. Anne Kettunen analyzed the data, interpreted the results and drafted the manuscript. Peer Berg wrote EVA. All authors read, revised and approved the final manuscript.

Data availability statement

Anonymized pedigree data is available upon request from the *Felagið Føroysk Ross*.

Conflict of interest

The authors declare no conflicts of interest.

References

- Berg, P., Nielsen, J., and Sørensen, M. K. (2006). EVA: realized and predicted optimal genetic contributions. In 8th World Congress on Genetics Applied to Livestock Production.
- Björk, E. A. (1984). *Færøsk bygderet 1ste del: Husbruget m.v.* (Torshavn: Matrikulstovan).
- Boichard, D., Maignel, L., and Verrier, E. (1997). The value of using probabilities of gene origin to measure genetic variability in a population. *Genetics Selection Evolution* 29(1). doi: <https://doi.org/10.1186/1297-9686-29-1-5>
- Caballero, A. and Toro, M. A. (2000). Interrelations between effective population size and other pedigree tools for the management of conserved populations. *Genet Res* 75(3), 331–374. doi: <https://doi.org/10.1017/s0016672399004449>
- Cervantes, I., Goyache, F., Molina, A., Valera, M., and Gutiérrez, J. P. (2011). Estimation of effective population size from the rate of coancestry in pedigreed populations. *J Anim Breed Genet* 128(1), 56–63. doi: <https://doi.org/10.1111/j.1439-0388.2010.00881.x>
- Duru, S. (2017). Pedigree analysis of the Turkish Arab horse population: structure, inbreeding and genetic variability. *Animal* 11(9), 1449–1456. doi: <https://doi.org/10.1017/s175173111700009x>
- Gandini, G. C., Bagnato, A., Miglior, F., and Pagnacco, G. (1992). Inbreeding in the Italian Haflinger horse. *Journal of Animal Breeding and Genetics* 109(1-6), 433–443. doi: <https://doi.org/10.1111/j.1439-0388.1992.tb00424.x>
- Giontella, A., Pieramati, C., Silvestrelli, M., and Sarti, F. M. (2019). Analysis of founders and performance test effects on an autochthonous horse population through pedigree analysis: structure, genetic variability and inbreeding. *Animal* 13(1), 15–24. doi: <https://doi.org/10.1017/s1751731118001180>
- Gutiérrez, J. P., Altarriba, J., Díaz, C., Quintanilla, R., Cañón, J., and Piedrafita, J. (2003). Pedigree analysis of eight Spanish beef cattle breeds. *Genet Sel Evol* 35(1), 43–63. doi: <https://doi.org/10.1186/1297-9686-35-1-43>
- Gutiérrez, J. P., Cervantes, I., and Goyache, F. (2009). Improving the estimation of realized effective population sizes in farm animals. *J Anim Breed Genet* 126(4), 327–359. doi: <https://doi.org/10.1111/j.1439-0388.2009.00810.x>
- Gutiérrez, J. P., Cervantes, I., Molina, A., Valera, M., and Goyache, F. (2008). Individual increase in inbreeding allows estimating effective sizes from pedigrees. *Genet Sel Evol* 40(4), 359–78. doi: <https://doi.org/10.1186/1297-9686-40-4-359>
- Gutiérrez, J. P. and Goyache, F. (2005). A note on ENDOG: a computer program for analysing pedigree information. *J Anim Breed Genet* 122(3), 172–

178. doi: <https://doi.org/10.1111/j.1439-0388.2005.00512.x>
- Hasler, H., Flury, C., Menet, S., Haase, B., Leeb, T., Simianer, H., Poncet, P. A., and Rieder, S. (2011). Genetic diversity in an indigenous horse breed: implications for mating strategies and the control of future inbreeding. *J Anim Breed Genet* 128(5), 394–406. doi: <https://doi.org/10.1111/j.1439-0388.2011.00932.x>
- Henryon, M., Ostensen, T., Ask, B., Sørensen, A. C., and Berg, P. (2015). Most of the long-term genetic gain from optimum-contribution selection can be realised with restrictions imposed during optimisation. *Genetics Selection Evolution* 47(1), 21–21. doi: <https://doi.org/10.1186/s12711-015-0107-7>
- Joensen, S. K. (2019). Activation of persistent *Streptococcus equi* subspecies *Zooepidemicus* in Faroese mares. Faculty of Science and Technology, University of Faroe Islands.
- Kettunen, A., Daverdin, M., Helfjord, T., and Berg, P. (2017). Cross-Breeding Is Inevitable to Conserve the Highly Inbred Population of Puffin Hunter: The Norwegian Lundehund. *PLOS ONE* 12(1), 170039–170039. doi: <https://doi.org/10.1371/journal.pone.0170039>
- Kvist, L., Niskanen, M., Mannerman, K., Wutke, S., Aspi, and J (2019). Genetic variability and history of a native Finnish horse breed. *Genet Sel Evol* 51, 35. doi: <https://doi.org/10.1186/s12711-019-0480-8>
- Lacy, R. C. (1989). Analysis of founder representation in pedigrees: Founder equivalents and founder genome equivalents. *Zoo Biology* 8(2), 111–123. doi: <https://doi.org/10.1002/zoo.1430080203>
- Lacy, R. C. (1995). Clarification of genetic terms and their use in the management of captive populations. *Zoo Biology* 14(6), 565–577. doi: <https://doi.org/10.1002/zoo.1430140609>
- Luís, C., Cothran, E. G., Mdo, O., and M (2007). Inbreeding and genetic structure in the endangered Sorraia horse breed: implications for its conservation and management. *J Hered* 98(3), 232–239. doi: <https://doi.org/10.1093/jhered/esm009>
- MacCluer, J. W., Boyce, A. J., Dyke, B., Weitkamp, L. R., Pfenning, D. W., and Parsons, C. J. (1983). Inbreeding and pedigree structure in Standardbred horses. *Journal of Heredity* 74(6), 394–399. doi: <https://doi.org/10.1093/oxfordjournals.jhered.a109824>
- Mancin, E., Ablondi, M., Mantovani, R., Pigozzi, G., Sabbioni, A., and Sartori, C. (2020). Genetic Variability in the Italian Heavy Draught Horse from Pedigree Data and Genomic Information. *Animals* 10(8), 1310–1310. doi: <https://www.mdpi.com/2076-2615/10/8/1310>
- Meuwissen, T. H. (1997). Maximizing the response of selection with a predefined rate of inbreeding. *J Anim Sci* 75(4), 934–974. doi: <https://doi.org/10.2527/1997.754934x>
- Mikko, S. T., Leivsson, C. G., and T (2004). Genetic diversity of the Faroe pony and relationships to other breeds. Poster in International Society for Animal Genetics (ISAG), Tokyo, Japan 11-16.9.2004.
- Nielsen, H. M. and Kargo, M. (2020). An endangered horse breed can be conserved by using optimum contribution selection and preselection of stallions. *Acta Agriculturae Scandinavica, Section A - Animal Science* 69(1-2), 127–130. doi: <https://doi.org/10.1080/09064702.2020.1728370>
- Olsen, H. F., Meuwissen, T., and Klemetsdal, G. (2013). Optimal contribution selection applied to the Norwegian and the North-Swedish cold-blooded trotter - a feasibility study. *J Anim Breed Genet* 130(3), 170–177. doi: <https://doi.org/10.1111/j.1439-0388.2012.01005.x>
- Onogi, A., Shirai, K., Amano, and T (2017). Investigation of genetic diversity and inbreeding in a Japanese native horse breed for suggestions on its conservation. *Animal Science Journal* 88(12), 1902–1910. doi: <https://onlinelibrary.wiley.com/doi/abs/10.1111/asj.12867>
- Perdomo-González, D. I., Sánchez-Guerrero, M. J., Molina, A., and Valera, M. (2020). Genetic Structure Analysis of the Pura Raza Español Horse Population through Partial Inbreeding Coefficient Estimation. *Animals (Basel)* (8), 10–10. doi: <https://doi.org/10.3390/ani10081360>
- Pérez-Enciso, M. (1995). Use of the uncertain relationship matrix to compute effective population size. *Journal of Animal Breeding and Genetics* 112(1-6), 327–332. doi: <https://doi.org/10.1111/j.1439-0388.1995.tb00574.x>
- Poyato-Bonilla, J., Perdomo-González, D. I., Sánchez-Guerrero, M. J., Varona, L., Molina, A., Casellas, J., and Valera, M. (2020). Genetic inbreeding depression load for morphological traits and defects in the Pura Raza Española horse. *Genetics Selection Evolution* 52(1), 62–62. doi: <https://doi.org/10.1186/s12711-020-00582-2>
- Sairanen, J., Nivola, K., Katila, T., Virtala, A. M., and Ojala, M. (2009). Effects of inbreeding and other genetic components on equine fertility. *Animal* 3(12), 1662–72. doi: <https://doi.org/10.1017/s1751731109990553>
- Solé, M., Valera, M., Gómez, M. D., Cervantes, I., and Fernández, J. (2013). Implementation of Optimum Contributions selection in endangered local breeds: the case of the Menorca Horse population. *J Anim Breed Genet* 130(3), 218–244. doi: <https://doi.org/10.1111/jbg.12023>
- Somogyvári, E., Posta, J., and Mihók, S. (2018). Genetic analysis of the Hungarian population of endangered Hucul horses. *Czech Journal of Animal Science* 63, 237–246.
- Vostrá-Vydrová, H., Vostrý, L., Hofmanová, B., Krupa, E., and Zavadilová, L. (2016). Pedigree analysis of the endangered Old Kladruber horse population. *Livestock Science* 185, 17–23. doi: <https://doi.org/10.1016/j.livsci.2016.01.001>



Conservation status of Creole sheep flocks in Brazil

Gilson Rudinei Pires Moreira

Department of Zoology, Institute of Biosciences, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil

Abstract: The Creole sheep (*Ovis aries*) is a transboundary breed that has been reared for centuries in southern Brazil, although it was officially recognized by the Brazilian authorities only in 2001. There are no updated local records on its current abundance, which is essential to establish conservation policies for the breed if required. Based on a survey conducted among farmers and considering all genealogical control records for Creole sheep provided by the Brazilian Sheep Breed Association (ARCO), we herein address the status of the breed in terms of the number and size of flocks, updating its geographical distribution. There are approximately 112 Creole flocks, totalling 8,844 reproductive ewes (flock size from 3 to 850 individuals; geometric average 54.77 individuals per flock). The flocks are primarily distributed in the southernmost states of Rio Grande do Sul and Santa Catarina, with sporadic occurrence in northern and central states (Rio de Janeiro, Minas Gerais and Goiás). The majority of the flocks ($n = 105$ flocks, with 8,298 ewes) are privately held, being reared primarily for meat and carpet wool production, similarly to other commercial breeds. A few flocks (5.61%) have remained with the same families, whose ascendants started rearing Creole sheep as an undefined breed over a century ago. However, over 65% of the current breeders have started rearing these sheep in the last two decades, following the official recognition of the breed and the foundation of the Brazilian Creole Sheep Breeder Association, which is in charge of breed promotion. Moreover, 73 flocks with genealogical control by ARCO have been established since the official recognition of the breed (total average = 427.80 specimens recorded per year). Recently, however, this number has decreased, with only 19 Creole breeders registering sheep in the last five years. Notwithstanding, the number of registered males remained stable (average of 78.2 rams/year, during 2016–2020). Taken together, the total number of the existing Creole ewes and males is well above the threshold adopted by the Food and Agriculture Organization of the UN (FAO) to qualify a given sheep breed as at risk ($< 80\%$ pure-breeding ewes).

Keywords: Genetic resources, Transboundary breeds, Sustainability, *Ovis aries*

Citation: Moreira, G. R. P. (2022). Conservation status of Creole sheep flocks in Brazil. *Genetic Resources* 3 (5), 68–74. doi: [10.46265/genresj.HFHG6814](https://doi.org/10.46265/genresj.HFHG6814).

© Copyright 2022 the Authors.

This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

The Creole sheep (*Ovis aries*) breed has been reared for centuries in the southernmost Brazilian states of Rio Grande do Sul and Santa Catarina, Uruguay and Argentina (Fernández, 2000; Vaz, 2000; Gonçalves *et al*, 2010; Peña *et al*, 2013; Silva *et al*, 2013b; Moreira *et al*, 2021). Presumably, the breed originated from the early sheep introduced by the European colonizers into the region in the 1600s (Henkes *et al*, 1993; Mernies *et al*, 2007; Peña *et al*, 2013). Until recently, Creole sheep were considered the third most abundant sheep breed in Argentina (Peña *et al*, 2013, 2015), although only a few flocks remain in Uruguay (Fernández, 2000). The Creole was the prevailing but uncharacterized sheep breed in southern Brazilian farms until the beginning

of the 20th century when it started being replaced by other commercial breeds (Hervé, 1922). Supposedly, in 1982, given the threat of extinction, a conservation programme for Creole sheep was initiated in southern Brazil by the Brazilian Agriculture Research Corporation (EMBRAPA) (Vaz, 2000). The remaining breeders were locally identified, variations in the phenotypes of their sheep were evaluated, and the establishment of new Creole flocks was initiated. To promote the breed, the Brazilian Association of Creole Sheep Breeders (ABCOC) was founded in 1999 and immediately requested official recognition for the breed. In 2001, based upon a standard broad enough to encompass the wide range of phenotypic variations existing in the corresponding flocks in southern Brazil at the time, Creole gained recognition as an official breed (Vaz *et al*, 2002, 2003).

Subsequently, the corresponding genealogical control of this newly recognized sheep breed was delegated to the Brazilian Association of Sheep Breeders (ARCO).

At the end of the last century, the number of Creole flocks was estimated to be 52 within the Rio Grande do Sul state, with approximately 5,000 individual sheep (Vaz, 2000). Since then, however, there have been no efforts to further examine the temporal changes in the number of existing flocks in general, except for those affiliated with ARCO. According to McManus *et al* (2014), the total number of such affiliated flocks was 65 at the end of the first decade of the present century. However, there is no numerical limit for the registration of a given flock. Also, in such a study, no information is given on the number of animals registered. Moreover, affiliation with ARCO is optional for the sheep breeders, and once registration starts, breeders can stop registration at any time while still maintaining the affiliation. According to the 'Domestic Animal Diversity Information System,' the breed is listed as "at-risk" ("vulnerable" category), based on the estimated population size of 5,422 individuals in 2018 (FAO, 2021). However, the process by which this inventory was conducted is not known. Thus, there is a lack of updated information on flocks that are actively supervised by the ARCO as well as the unsupervised flocks. Unsupervised flocks are thought to be more numerous than those under ARCO's supervision. This population information is important not only to monitor the numerical performance at the spatiotemporal scales but also to propose future conservation policies for the breed, if necessary.

Creole sheep are medium-sized animals, with the smallest body size among other commercial breeds reared in the region. Morphometric data provided by Mernies *et al* (2007) and Moreira *et al* (2021) indicate that meat production is the main attribute, rather than milk production. Meat production may be an important commercial alternative for this rustic, behaviorally active and naturally adaptive sheep. Although not evaluated in terms of the organoleptic properties, Creole sheep meat is traditionally recognized as being tasty and light. Creole sheep can be recommended for low-tech breeding systems, as the animals are adapted to mountainous areas, where comparatively sparse quality pastures grow (Fernández, 2000; Mernies *et al*, 2007; Peña *et al*, 2013; Moreira *et al*, 2021). Recent studies have highlighted that Creole lambs finished in feedlots responded satisfactorily to low-cost concentrates, such as the by-products of the rice industry, while still producing high-quality carcasses when supplied with dressing percentages varying from 46.36% to 53.26% (Matos, 2016; Oliveira, 2016). In addition to purebred lambs, progeny obtained by crossing the F1 animals with other commercial breeds (e.g. Suffolk) and reared on pasture reached a suitable size for slaughtering, since relatively small sheep carcasses (14–16 kg) are currently in demand in the region (Matos, 2020, 2021). Overall, the preliminary observations indicate that such aspects that

are supported by the ABCOC affiliates are turning Creole into a more popular breed, leading to a substantial increase in the number of animals.

In the present study, changes in the population size of this sheep breed are analyzed in space and time. A survey among Brazilian Creole sheep breeders first listed and mapped the currently active Creole flocks in the country. This was followed by documenting the variations in the age of the flocks and the number of individuals per flock. Finally, the Creole flock book records were assessed to explore variations in the number of registered animals throughout the existence of Creole as an official breed.

Material and methods

A general survey was conducted between June and July 2021. The ABCOC data bank was used to identify Creole breeders, to whom two questions were individually asked in formal interviews: (1) For how long have you (or your ancestors) been continuously breeding Creole sheep, independently of being genetically controlled or not? (2) What is the number of ewes present in your Creole flock at present? A list of such flock owners (or managers), along with the size of their flocks and municipalities, is presented in Supplemental Data 1. Their phone numbers and farm names are available at www.ovelhacrioula.com.

Data for the genealogy-controlled flocks were retrieved from the ARCO data bank. Specifically, these data correspond to the annual records for all Creole sheep breeders who have been consistently affiliated with ARCO throughout the official existence of the breed (2001–2020) (Supplemental Data 2). For such flocks, both sex and pedigree ranks were included in the census. According to ARCO's rule applied to local breeds, the individuals of a given non-controlled flock are initially registered within a specific category called the 'Brazilian Genealogical Registration' (RGB). The non-affiliated individuals that meet the phenotypic breed standard are registered for the first time as RGB_{base}. Such individuals only reach purebred status (PO) after continuous ARCO-controlled breeding until the fifth generation, that is, after passing across the RGB₁ to RGB₄ categories. In the case of Creole sheep, the first PO animals appeared in 2005. However, RGBs are being registered even today, because the breed flock book remains open; that is, Creole is still under development and is still open to the inclusion of previously unregistered but typical sheep.

Results and Discussion

The survey retrieved 112 Creole flocks, totalling 8,844 reproductive ewes (Supplemental Data 1). The number of ewes per flock varied from 3 to 850 (geometric mean = 54.77). The corresponding frequency distributions were biased toward small numbers, with over 50% of the flocks comprising fewer than 40 individuals (Figure 1A). Creole flocks are primarily distributed in Rio Grande do Sul (76%) and Santa Catarina (22%) states, with

sporadic records in Paraná, São Paulo, Rio de Janeiro, Minas Gerais, and Goiás (Figure 2). Flocks varied in age from 1 to 180 years (Figure 1B). A few flocks ($n = 6$) were centenarians, whereas over 65% of flocks were below 20 years of age, with approximately half ($n = 31$) of the latter being younger than 5 years. The majority of the flocks were maintained by private owners (93.75%), and the remaining few were owned by public agencies, including federal, state or municipal institutions involved in research and/or education.

Compared with the original data reported by Vaz (2000) at the end of the last century, the number of Creole flocks is more than double at present, although the average size of each corresponding flock is approximately half. Although the precise reasons underlying this trend remain unknown, it is likely explained by an overall loss in the value of sheep breeding in the region, which has been greater than that of other rural activities, such as soybean cultivation and forest plantations. Silva *et al* (2013a) demonstrated that sheep breeding in the Rio Grande do Sul qualifies as the secondary exploitation of farms, with small flock size and subsistence being the primary objective. The exponential increase in the number of Creole flocks, resulting primarily from private investment, demonstrates the importance of the development activities exercised by ABCOC and public institutions in attracting new breeders. For instance, ABCOC has effectively encouraged breeders to participate in the major farm fairs in the region, such as EXPOINTER, which is held annually in the Esteio municipality of Rio Grande do Sul and is considered to be the largest livestock show in Latin America (Moreira *et al*, 2021). ABCOC has prioritized activities aimed at garnering public attention to breed rusticity and the associated breeding attributes and cultural significance as well as the various Creole products, such as high-quality meat and long-staple, colourful wool specifically for use in the production of carpets and other handcrafts (Matos, 2020, 2021; Trierweiler, 2021).

According to the Food and Agriculture Organization of the UN (FAO, 2013), there must be a minimum of 7,200 breeding females to qualify a given sheep breed as being not at risk. Currently, the number of breeding Creole ewes within the Brazilian borders is above this threshold. Moreover, there has been an exponential increase in the number of flocks and expansion in their distribution to the states further north. As such, most of the young flocks have been established recently with specimens from southern Brazil (Moreira *et al*, 2021). Importantly, Creole is a transboundary breed (FAO, 2015), with a relatively high number of flocks in Argentina and Uruguay, as mentioned above. Those flocks are not considered in the present study. Thus, from the viewpoint of population size, the breed is far above the risk threshold in a broad geographic sense. However, other factors should also be considered when determining the degree of endangerment from a conservation perspective of a given breed, such

as localized distribution (Carson *et al*, 2009). Many Brazilian flocks are located far apart from one another because this sheep breed is widespread in southern parts of South America. As a result, the Creole may eventually face the risk of local extinction (McManus *et al*, 2014), albeit with a low impact on the general integrity of the breed. Although not taken into account in our survey, we estimated the total number of reproductive rams existing among the overall Brazilian Creole flocks to be ca. 177, since the ratio of one adult ram to 50 reproductive ewes is expected for the breed (Moreira *et al*, 2021). In other words, results suggested the number of reproductive males also goes beyond the defined thresholds established by FAO. However, these data have been retrieved from non-registered flocks, falling into the '< 80% pure-breeding ewes' category established by FAO (2013), and thus the number of registered rams should also be considered to determine the risk status for Creole sheep, as discussed further below.

The cumulative number of Creole breeders affiliated with ARCO throughout the existence of the breed was 73 (Supplemental Data 2). The number of flocks registering sheep in the book varied from 6 to 25 per year (mean = 13.6 per year). The total number increased in the initial years since recognition, reaching a peak in 2009 (Figure 3A, Supplemental Data 2), but it declined progressively until 2014, following a plateau thereafter until the present time. The cumulative number of Creole animals registered by the ARCO has reached 8,918 in two decades (Supplemental Data 2). The total number of newly registered animals varied from 137 to 828 per year. The distribution of this number over time (Figure 3B) followed a consistent trend, varying proportionally with the number of flocks registered in each year ($y = 34.61x - 43.6$; $n = 20$; $r = 0.83$; $p < 0.001$). This pattern can be explained by the balance between the number of Creole breeders entering and leaving the registration over time. The number of breeders who started registering their flocks at ARCO continued to increase progressively (Figure 3C), albeit with a simultaneous increase in the number of breeders with interrupted registration (Figure 3D). Only 19 breeders have consistently registered one or more animals in the flock book in the last five years. Of the 73 flocks with animals that were registered with ARCO, only 32 (28.57%) were active with certainty (Supplemental Data 2), among the list of flocks presented in Supplemental Data 1. That is, approximately two-thirds of the corresponding flocks either do not exist anymore or information on whether they are active at present is missing. Of note, however, there has been an increase in the proportion of males registered; as such, rams accounted for 391 records in the last five years (average = 78.2 rams registered per year; Supplemental Data 2). It is worth also mentioning that EMBRAPA registered many more animals than anyone else, which may lead to a narrowing of genetic participation in the next generations. If this is flowing

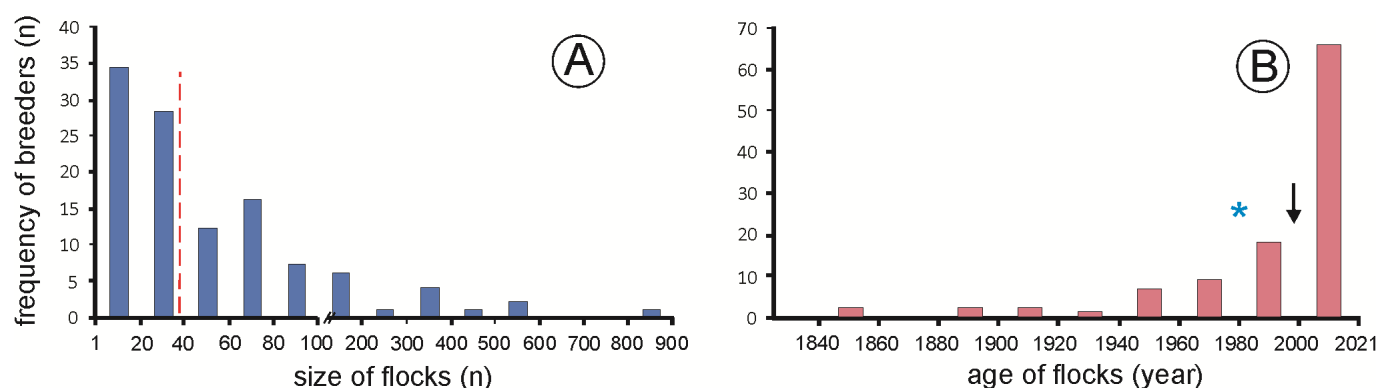


Figure 1. Frequency variations in the number of ewes per flock (A) and the year of Creole sheep flock creation (B) in Brazil, based on a survey among Creole sheep breeders conducted from June to July 2021. A list of breeder names and municipality/state, age, and size of the corresponding flocks are provided in [Supplemental Data 1](#). The dashed line (A), asterisk, and arrow (B) indicate, respectively, the statistical median, the beginning of Creole sheep conservation programme by the Brazilian Agriculture Research Corporation (EMBRAPA), and the foundation of the Brazilian Association of Creole Sheep Breeders (ABCOC).

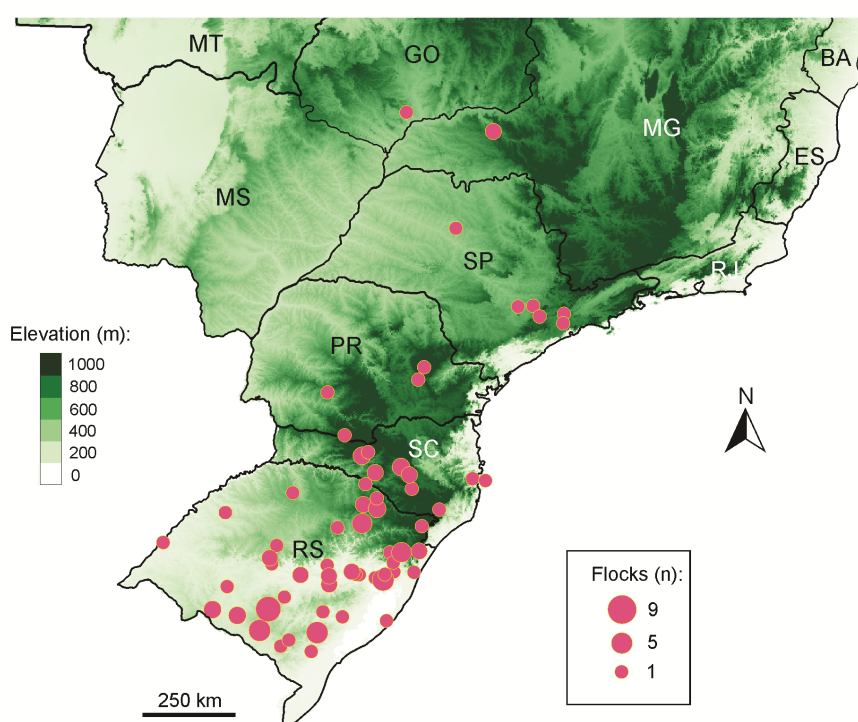


Figure 2. Geographic distribution of Creole sheep flocks in Brazil ($n = 112$). BA, Bahia; ES, Espírito Santo; GO, Goiás; MG, Minas Gerais; MT, Mato Grosso; MS, Mato Grosso do Sul; PR, Paraná; RJ, Rio de Janeiro; RS, Rio Grande do Sul; SC, Santa Catarina; SP, São Paulo. Farms were distributed according to municipalities, a list of which is presented in [Supplemental Data 1](#). The corresponding geographic coordinates were obtained from [Falling Rain Genomics \(2007\)](#).

out into the unregistered flocks, then there may be a problem in that they are being absorbed and replaced rather than contributing to the maintenance of the genetic resource, which should be further explored.

Registration is fundamental in a given conservation programme because it acts as a continuous filter to maintain the breed's integrity by excluding animals that are used for crossing with other breeds as well as the newborns that are not eligible for registration. As already pointed out by [Alderson \(2009\)](#), registration also captures the eagerness and conviction of breeders

for the breed they farm. By being associated with rural traditions and the history of many families in the region, as demonstrated by the old age of several flocks estimated herein, Creole breeders are usually committed to the conservation of this breed and are passionate and proud of it. In this context, the decrease in registration may be attributed in part to the death of the owners, following which the successor family members did not continue the flocks. Others may have simply changed their preference, quitting Creole breeding and starting to rear a different breed. Particularly in the northeast of

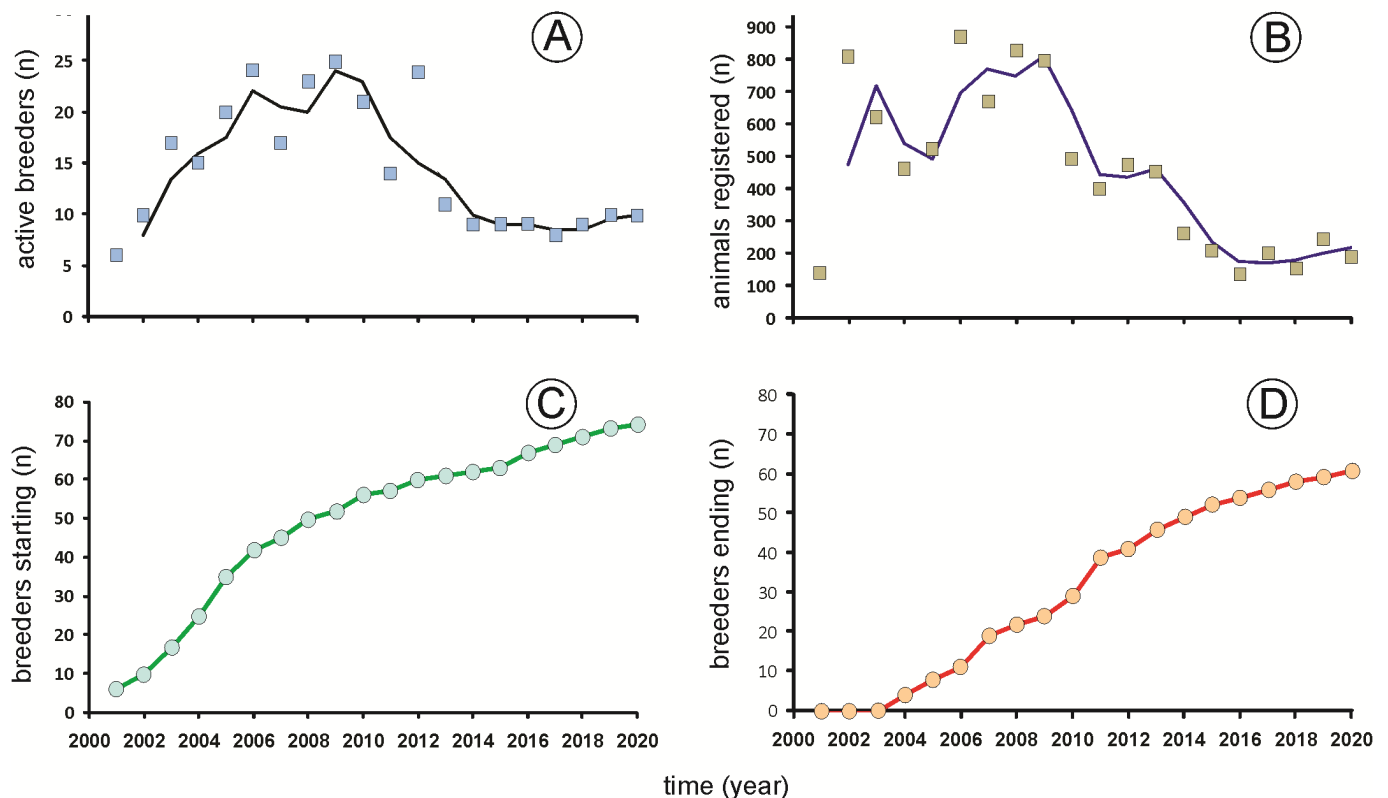


Figure 3. Annual variations in the number of Creole sheep breeders (A) and animals per flock (B) registered annually at the Brazilian Sheep Breed Association (ARCO) in the present century, and the cumulative number through the period of breeders starting (C) and ending (D) registration in the flock book. Breeders specifically affiliated with ARCO, along with the number of sheep registered each year in the Creole flock book, are listed in [Supplemental Data 2](#).

Rio Grande do Sul, several breeders have likely stopped sheep breeding in general due to the harm caused by the attacks of cougars, wild boars and domestic dogs. However, many argue that registration does not pay off because ARCO's costs are relatively high and the Creole is a low-price sheep. Thus, breeders simply stop registering their sheep after a certain time of affiliation with ARCO, and most never register them. From a conservation perspective, this decrease has somewhat attenuated since the proportion of males registered remains high, being well above the threshold of 35 males adopted by [FAO \(2013\)](#) (< 80% pure-breeding ewes) for species of low reproductive capacity, such as sheep. As already mentioned, the Creole breed predates such records provided by ARCO. Given the relatively low registration numbers, it remains unknown whether the Creole flock book really captures the breed and its genome, or whether most of this lies outside of the official records.

Conclusions

The population size of the Brazilian Creole sheep has increased substantially in the present century, surpassing the threshold established by [FAO \(2013\)](#) for endangered sheep breeds worldwide; thus, Creole does not qualify as an 'at-risk' breed ('vulnerable' category). The breed

is primarily reared in the states of Rio Grande do Sul and Santa Catarina, with few flocks in the northern and central parts of Brazil. This surge was achieved following the initiation of a conservation programme for Creole by EMBRAPA in 1982 and the foundation of ABCOC in the late last century. The resulting efforts were followed by the official recognition and beginning of the genealogical control of the breed conducted by ARCO in the early present century. This is an example of how public institutions and private farmers can join efforts to successfully save a sheep breed from extinction and substantially improve its population size within a few decades. Thus, as a breed that is not a risk, Creole sheep warrant further improvement as a breed [FAO \(2015\)](#).

Supplemental Data

Supplemental Data 1: Breeder names and municipality/state, age (years since the flock creation), and size (number of ewes) of the corresponding Creole sheep flocks between June and July 2021.

Supplemental Data 2: Number of animals registered by breeders per year in the Creole sheep flock book from 2001 to 2020 at the Brazilian Association of Sheep Breeders (ARCO), including both the Brazilian Genealogical Registration (RGB) and Purebred (PO) categories.

Acknowledgments

I am grateful to the ABCOC members for kindly participating in the survey of Creole flocks and to ARCO for supplying data available on the historical registration records of the breed. I thank Julia Fochezato (UFRGS) for creating the map. I am also grateful to Gregoire Leroy and three anonymous reviewers whose suggestions improved substantially the first version of the manuscript. This work was supported by a CNPq research fellowship.

Conflict of interest statement

The author declares that no conflict of interest exists.

References

- Alderson, L. (2009). Breed at risk: Definition and measurement of the factors which determine endangerment. *Livestock Science* 123, 23–27. doi: <https://doi.org/10.1016/j.livsci.2008.10.001>
- Carson, A., Elliott, M., Groom, J., Winter, A., and Bowles, D. (2009). Geographical isolation of native sheep breeds in the UK - Evidence of endemism as a risk factor to genetic resources. *Livestock Science* 123, 288–299. doi: <https://doi.org/10.1016/j.livsci.2008.11.026>
- Falling Rain Genomics (2007). Global Gazetteer ver. 2.3, 1996–2017. url: <http://www.fallingrain.com/world/BR>.
- FAO (2013). In vivo conservation of animal genetic resources volume 14 of *FAO Animal Production and Health Guidelines*. url: <https://www.fao.org/3/i3327e/i3327e00.htm>.
- FAO (2015). The Second Report on the State of the World's Animal Genetic Resources for Food and Agriculture, ed. Scherf, B. D. and Pilling, D. FAO Commission on Genetic Resources for Food and Agriculture Assessments, (Rome) 562p. doi: <https://doi.org/10.4060/I4787E>
- FAO (2021). Domestic animal diversity information system. url: <http://www.fao.org/dad-is/browse-by-country-and-species/en/>.
- Fernández, G. (2000). Situación de los recursos genéticos domésticos locales del Uruguay. *Archivos de Zootecnia* 49, 330–340.
- Gonçalves, G. L., Moreira, G. R. P., Freitas, T. R. O., Hepp, D., Passos, D. T., and Weimer, T. A. (2010). Mitochondrial and nuclear DNA analyses reveal population differentiation in Brazilian Creole sheep. *Animal Genetics* 41, 308–310. doi: <https://doi.org/10.1111/j.1365-2052.2009.01986.x>
- Henkes, L. E., Weimer, T. A., Franco, M. H. L. P., and Moraes, J. C. F. (1993). Genetic characterization of the Crioula Lanada sheep from Southern Brazil. *Revista Brasileira de Genética* 16, 449–455.
- Hervé, E. (1922). Pecuária-agricultura: Ensino de agronomia e veterinária. In *O Rio Grande do Sul*, ed. Costa, A. R., (Porto Alegre: Editorio Globo), volume 1, 27–40.
- Matos, A. J. (2016). Ganho de peso de cordeiros da raça ovina Crioula em sistema de confinamento com utilização de farelo de arroz integral. Universidade Federal do Pampa, Dom Pedrito. Unpublished Bachelor's Thesis.
- Matos, A. J. (2020). Cordeiro Crioulo surpreende no concurso de carcaças da Agrovino. *Revista Arco* 25, 55–56.
- Matos, A. J. (2021). Cordeiro cruza Crioula-Suffolk vence na categoria “Cruzamentos Orientados” no concurso de carcaças da Agrovino. *Revista Arco* 28, 34–34.
- McManus, C., Hermuche, P., Paiva, S. R., Moraes, J. C. F., Melo, C. B., and Mendes, C. (2014). Geographical distribution of sheep breeds in Brazil and their relationship with climatic and environmental factors as risk classification for conservation. *Brazilian Journal of Science and Technology* 1(3). doi: <https://doi.org/10.1186/2196-288X-1-3>
- Mernies, B., Macedo, F., Filonenko, Y., and Fernández, G. (2007). Índices zoométricos en una muestra de ovejas criollas uruguayas. *Archivos de Zootecnia* 56, 473–478. url: <https://www.redalyc.org/pdf/495/49509918.pdf>.
- Moreira, G. R. P., Maestri, R., Garcia, B. V., Freitas, T. R. O., and Gonçalves, G. L. (2021). *Raça ovina crioula: Variação morfológica e classificação em vinte anos de Expointer* (Porto Alegre: Forma Diagramação), 214pp.
- Oliveira, M. G. (2016). Características quantitativas e qualitativas da carcaça de cordeiros da raça ovina Crioula em confinamento. Universidade Federal do Pampa, Dom Pedrito. Unpublished Bachelor's Thesis.
- Peña, S., Lopez, G., Martínez, R., Abbiati, N., Castagnasso, E., Giovambattista, G., and Genero, E. (2013). Características zoométricas de ovinos criollos de cuatro regiones de la Argentina. *Actas Iberoamericanas de Conservación Animal* 3, 174–181. url: <https://aicarevista.jimdo.com/n/%C3%BAmeros/vol/%C3%BAmen-3-2013/>.
- Peña, S., López, G. A., Abbiati, N., Género, E., and Martínez, R. (2015). Avances en la caracterización zootécnica de la raza ovina Criolla Argentina. *Revista de Divulgación Técnica Agropecuaria* 2, 11–20. url: <https://revistafcaunlz.gramaweb.com.ar/2015/03/>.
- Silva, A. P. S. P., Santos, D. V., Kohek, I., Machado, G., Hein, H. E., Vidor, A. C. M., and Corbellini, L. G. (2013a). Ovinocultura do Rio Grande do Sul: Descrição do sistema produtivo e dos principais aspectos sanitários e reprodutivos. *Pesquisa Veterinária Brasileira* 33, 1453–1458. doi: <https://doi.org/10.1590/S0100-736X2013001200010>
- Silva, M. C., Lopes, F. B., Vaz, C. M. S., Paulini, F., Montesinos, I. S., Fioravanti, M. C. S., Mcmanus, C., and Sereno, J. R. B. (2013b). Morphometric traits in Crioula Lanada ewes in Southern Brazil. *Small Ruminant Research* 110, 15–19. doi: <https://dx.doi.org/10.1016/j.smallrumres.2012.09.002>

- Trierweiler, A. C. (2021). O coxinilho. *Revista Arco* 29, 66–67.
- Vaz, C. M. S. L. (2000). Morfologia e aptidão da ovelha crioula lanada. url: <http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/227035>.
- Vaz, C. M. S. L., Medeiros, F. P., and Moreira, G. R. P. (2002). Padrão racial da ovelha crioula. *Boletim Informativo ABCOC* 1, 2–5.
- Vaz, C. M. S. L., Moreira, G. R. P., and Caon, J. E. M. A. (2003). O registro de uma raça: Da ovelha crioula à homologação da raça ovina crioula. *Boletim Informativo ABCOC* 4, 5–8.