Diversity Analysis among Ethiopian Fenugreek (*Trigonella foenum-graecum* L.) Accessions Based on Morpho-agronomic Traits

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Authors' contributions

This work was carried out in collaboration among all authors. Author GWT drafted the manuscript, supervised the fieldwork and collected data, and conducted the analysis of variance, cluster and principal component analysis. Authors DFL and DTG participated in the fieldwork, designing, and reviewing of the manuscript. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

A field experiment was carried out in the meher season (July–November 2021 and 2022) at Kulumsa, Southeastern Ethiopia. Genetic diversity study among the available genetic resources is the most important step in improving crops through selection or hybridization. The aim of this study is to determine the magnitude of diversity among Ethiopian fenugreek accessions based on morpho-agronomic traits. Twenty-eight Ethiopian fenugreek accessions were arranged in a 4×7 alpha lattice design with three replicates. Fifteen morpho-agronomic traits of the fenugreek accessions showed highly significant differences (p≤0.01)) in the pooled analysis of variance. The

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first three principal components contributed 83.1% of the genotypic variation. The number of fertile pods plant-1, days to 50% flowering, number of primary branches plant-1, seed yield and number plant-1, and plant height are the most contributing characters for the total variation. The accessions were clustered into three; the first, second, and third accounted for 11, 6, and 11 accessions, respectively. Accessions grouped under cluster I was characterized by a late grain-filling period, a high primary branches plant^{-1}, a long pod length, and a higher number of seeds plant^{-1}. The second cluster accessions were early emerging but flowered and matured late, with short pod length and low yielders; however, cluster III accessions were high yielders, with higher fertile pods, seeds, and seed yield plant^{-1}. The highest inter-cluster distances were observed between the I and III and I and II clusters. The first cluster was mostly responsible for the particularly heterotic returns and segregants that might result from hybridization between accessions included in this cluster. The findings have practical implications for breeding programs and stakeholders who are going to exploit the advantage of each accession in each cluster. The phenotypic and genotypic correlation, direct and indirect effects of the traits and the genetic variability and advance from selection for those accessions needs to be checked for inclusion in the next breeding program.

1. INTRODUCTION

"Fenugreek (*Trigonella foenum-graecum* L) is an annual, self-pollinating, diploid (2n=16), legume plant species" (Moradi et al., 2010). "It is native to the Mediterranean's eastern shores and is widely cultivated in different parts of the world, mainly in India, Egypt, Ethiopia, and Morocco, and occasionally in England" (Sadeghzadeh-Ahari et al., 2010).

"Ethiopia is also identified as the homeland of fenugreek subspecies Mediterranean, ecotype Abyssinians, with its distribution covering Eritrea and Somalia" (Sinskaya, 1950). Fenugreek cultivation and production started many years ago, in different part of the country as a major cash crop, mainly in highland areas similar to other highland legume crops (Fikreselassie et al., 2012; Getu and Hirko, 2022). Fenugreek is sown at the end of the *Meher* season depending on rainfall distribution as the crop can grow using residual soil moisture and prefers well-drained black (alluvial) soil (Petropoulos, 2002). According to the central statistical agency report from 2019/20, the 27,595.21 ha of land was dedicated to fenugreek from 381,046 peasant holdings with an approximate production and productivity of 37672.87 tons and 1.37 ton ha^{-1}, respectively (CSA, 2021). In the Arsi highlands, fenugreek was planted on 2,847.13 ha of land on 17,120 peasant holdings. The total production was approximately 3990.0 tons, with productivity exceeding the national average (1.4 ton ha^{-1}) (CSA, 2021).

Fenugreek leaves are composed of 6.0% carbs, 4.4% protein, 0.9% lipids, 1.5% minerals, 1.1% fiber, and 86.1% moisture. According to Rao (2003), it contains calcium, phosphorus, iron, carotene, riboflavin, niacin, and vitamin C. The seed has 44.1% carbohydrates, 13.7% moisture, 26.2% protein, 5.8% fat, 3.0% mineral, and 7.2% fiber (Wani and Kumar, 2018). Among the earliest medicinal herbs is fenugreek; its leaves, seeds, and even the entire plant have all been utilized for therapeutic purposes (Sinskaja, 1961). Extracts from powdered fenugreek seeds are used to treat wounds, skin irritations, lice, and excessive blood sugar, among other conditions (Acharya et al., 2006). Lowering blood sugar and cholesterol levels is possible with fenugreek seed extract as an anti-diabetic (Al-Habori and Raman, 2002). To treat skin infections and irritation, fenugreek seed extracts can be used to manufacture ointments or beverages to lower fever and menstrual cramps (Meghwal and Goswami, 2012). A common way to add a maple taste to candies and confections is to utilize ground seeds. When taken internally, fenugreek helps with sinus issues, respiratory issues, coughing, and bronchitis (Betty, 2008). According to Meghwal and Goswami (2012), fenugreek, like the majority of Trigonella species, has potent antifungal properties and may be utilized as an insect repellant. South and Central Asian nations have utilized the green leaves and both ripe and unripe seeds as sustenance (Petropoulos, 2002). The majority of the time, fenugreek is used to enhance low-protein diets like cereal crops because of its high protein content (43.8 g/100 g) (Naidu et al., 2011). Fenugreek enriches soil through symbiosis with microorganisms fixing atmospheric nitrogen (Bromfeild et al., 2001; Ahari et al., 2009).

In Ethiopia, the crop is produced either as a seed spice, food, or for other special purposes, such as for nursing mothers and infants as a breakfast. It is a source of income, a flavoring of traditional bread, and maintains the soft texture of "*tef-injera*" (Engles et al., 1991). Arsi farmers use fenugreek flour, deepening it in water at night and removing the water in the morning for drinking. In Hararghe, it is used for preparation of "*Lafiso*", which is made from either maize or sorghum "*injera*" and served for baking in a mixture of fenugreek flour boiled either with meat or alone (Roba and Simion, 2022). Though fenugreek is an essential crop, it is unnoticed and underutilized, which may inadvertently risk losing significant landraces and information used by farmers that have been preserved and utilized by farmers over many years (Fikreselassie et al., 2012).

More than nine varieties have been registered and are under production for specified and wider cultivation across the country. Those varieties were developed from selection of available genetic resources collected from different corners of the country through the involvement of the federal and regional research institutes. Different scholars explored copious research findings in the country; including genetic diversity, genetic variability, association of traits, multi-environment evaluation, importance, disease resistance, variety registration, and fertilizer recommendation (Fikreselassie, 2012; Fikreselassie et al., 2012; Fikreselassie et al., 2013; Wojo et al., 2016; Serbessa et al., 2019; Abtew and Abate, 2020; Bekele et al., 2020; Melese et al., 2020; Roba et al., 2022; Roba and Simon, 2022; Beze and Shumbulo, 2024; Roba and Mohamed, 2024; Urgi et al., 2024).

Information available about fenugreek landraces especially the genetic diffrences, variability, the association of morpho agronomic traits, optimum planting time is limited. In addition, the most important quantitative traits contributed to the total variation, the genetic differences of land races based on marker assisted selection are neither complete nor conclusive. Thus, it is indispensable to generate information on the magnitude of genetic diversity based on principal component and distance analysis. Therefore, this experiment was commenced with the aim to study the diversity among Ethiopian fenugreek accessions based on morpho-agronomic traits in Ethiopia.

2. MATERIALS AND METHODS

2.1 Description of Experimental Site

In Meher (July–November 2021 and 2022), the experiment was carried out under field condition at the Kulumsa Agricultural Research Center (KARC) in southeastern Ethiopia. It is located in the Tiyo District Arsi Administrative Zone of Oromia Regional State, 167 kilometers southeast of Addis Ababa, at an elevation of 2210 meters above sea level. KARC is situated between 8° 00' and 8° 02'N and 39° 07' and 39° 10'E. Slopes range from 0% to 10% on the mild terrain where the center is situated. Between 1,980 and 2,230 meters, its height varies very little (Abayneh et al., 2003). The region has an unequal rainfall regime with a rainy season that lasts from March to September. The agro-climatic conditions are humid, with an average annual rainfall of 832 mm. The typical annual minimum and maximum temperatures are 10°C and 23.20°C, respectively, with July and August being the high rainfall months. The three primary soil types found in the center are Vertic Cambisol, Vertic Luvisol, and Eutric Vertisol (Abayneh et al., 2003).

2.2 Experimental Materials and Design

One hundred fenugreek accessions in total were originally obtained from Ethiopian Biodiversity Institute, which were collected from different parts of the country in altitude ranges of 1700-2800 m.a.s.l.; selected twenty six advanced lines were utilized in this study (Table 1). Two varieties (Chala and Burka) were acquired from the DebreZeit Agricultural Research Center and utilized as standard checks.

The trial field was mechanically cleared, properly plowed, and harrowed. The experimental plots were leveled with a hand hoe. Alpha lattice was the experimental design. Three replications, four blocks, and seven fenugreek accessions per block were used in the setup. The distances between plots, blocks, and replications for each unit plot were 0.6m, 1m, and 1.5m, respectively. The plot was 3.6m^2 and was 2m long by 1.8 m wide. The seeds were drilled at a rate of 25 kg ha^{-1}, with a 0.3m gap between rows. 100 kg ha^{-1} of NPS fertilizer was applied at the time of planting. The total harvestable net size was 2.4m^2. Hand weeding and cultivation was employed to control weeds at 15 days intervals and when needed. Harvesting and threshing were done manually.

2.3 Data Collection

Plot and plant-level data were collected. Ten randomly chosen plants from each plot's middle four rows had their morpho-agronomic characteristics noted. These characteristics include seed yield, yield-related characteristics, phenological data, and agronomic data. According to the crop descriptor, phenological data is collected for the following times: days to 50% emergency, days to 50% blooming, days to 90 physiological maturity, and grain filling periods. Agronomic variables include plant height (cm), number of primary branches, number of fertile pods plant^{-1}, and pod length (cm). On the other hand, yield and yield-related traits are recorded as seed yield (kg ha^{-1}), thousand seed yield (g), number of seeds pod^{-1}, number of seeds plant^{-1} (g), number of seeds plant^{-1}, biological yield plot^{-1}, and harvest index (%).

2.4 Statistical Analysis

Using R software (4.3.1 version) (Anonymous, 2023), the analysis of variance was calculated on the data gathered for the fifteen morpho-agronomic features, following the procedures outlined in Gomez and Gomez (1984).

The pooled mean values of the genotypes of the two cropping seasons were subjected to the procedures of multivariate analyses. However, the traits considered in this study did not share a common measurement unit and apparatus; the morpho-agronomic traits for the principal component (PCA), hierarchical agglomerative cluster, and inter-cluster and intra-cluster distance analyses (standardized Mahalanobis's D^2) were all standardized to a mean of zero and a standard deviation of one (Manly and Manly, 1994; Mohammadi and Prasanna, 2003; Sneath, 2005). Random number generation and statistical computations were performed using the R Stats Package (Anonymous, 2023). The cluster distances were shown using the "clv" program (Nieweglowski, 2023). Using the Euclidean distance metric and Ward D^2's cluster analysis approach, the NbClust software package was used to determine the optimal number of clusters through graphical presentation (Charrad et al., 2014).

Table 1. List of the Fenugreek genotypes with their passport data

Genotypes Name	Collection region	Status
237984	Oromia	Advanced lines
201609	NA	Advanced lines
201637	NA	Advanced lines
202143	NA	Advanced lines
216900	Oromia	Advanced lines
219509	Oromia	Advanced lines
53015	SNNP	Advanced lines
201604	Amhara	Advanced lines
FG-53072	Amhara	Advanced lines
FG-53007	Amhara	Advanced lines
FG-20	Oromia	Advanced lines
FG-38	Oromia	Advanced lines
FG-83	Oromia	Advanced lines
FG-73	Oromia	Advanced lines
FG-53049	Amhara	Advanced lines
FG-5	Oromia	Advanced lines
FG-10	Hararghe	Advanced lines
FG-1	Oromia	Advanced lines
FG-8	Oromia	Advanced lines
FG-2	Oromia	Advanced lines
FG-11	Oromia	Advanced lines
FG-4	Oromia	Advanced lines
FG-3	Oromia	Advanced lines
FG-7	Oromia	Advanced lines
FG-12	Oromia	Advanced lines
FG-6	Oromia	Advanced lines
Chala	-	Variety
Burka	-	Variety

NA = not identified

PCA was performed to identify the traits that most significantly influenced overall variance and the classification of Ethiopian fenugreek genotypes. The variance proportions explained by the scree plot of the most important dimensions and variable PCA through dimension reduction techniques were displayed according to Cattell (1966). The 'factoextra' package was used for extracting and visualizing the results of multivariate data analyses (Kassambara and Mundt, 2020).

To analyze the assembling pattern of 28 fenugreek accessions, they were grouped into distinct clusters based on the mean values of 15 morpho-agronomic variables using the squared Euclidean distance approach and ward linkage. The genetic distance between and within clusters was determined using Mahalanobis' (1936) generalized distance statistics according to the following formula. The inter and intra cluster-cluster distance analyses (standardized Mahalanobis's D^2) were based on the mean data of standardized to mean zero and standard diviation of one. The values determined between cluster pairs were regarded as chi-square values and were examined for significance using p-degrees of freedom, where "p" denotes the number of traits employed.

$$D^2_{ij} = (xi-xj)'cov^{-1}(xi-xj)$$

Where: D^2_{ij}= the square distance between cases I and J, xi and xj = vectors of the values of the variables for cases I and J, and Cov^{-1} = the pooled within groups variance covariance matrix.

3. RESULTS AND DISCUSSION

3.1 Analysis of Variance

The combined analysis of variance showed highly significant differences ($p≤0.01$) in plant height, days to 50% emergency, days to 50% flowering, days to 90% physiological maturity, biological yield, seed yield per hectare and plant, thousand seed weight, grain filling period, primary branch, number of fertile pods per plant, pod length, number of seeds per plant, pod length, number of seeds per pod, and harvest index among the fenugreek accessions studied (Table 2). Previous research has also documented notable morpho-agronomic variations in these characteristics among Ethiopian fenugreek accessions other than those in this study (Fikreselassie et al., 2013; Wojo et al., 2016; Bekele et al., 2020; Melese et al.,

2020; Roba et al., 2022; Roba and Mohamed, 2024), which is in line with our findings. The results of this experiment were in agreement with the reports of Sadeghzadeh-Ahari et al. (2010); Gurjar et al. (2016); Yadav et al. (2017); Meena et al. (2019); Choudhary et al. (2022); Kumar et al. (2023).

The studied fenugreek accessions showed a high degree of variation, which implies that they might be a useful source of genetic material for future fenugreek breeding programs. A significant interaction between accessions and year was found, suggesting that the accessions responded differently to traits like days to 50% flowering, grain filling period, thousand seed weight, plant height, pod length, biological yield, and number of seeds per pod[-1] over the course of the two years. The significance of choosing suitable fenugreek accessions for certain breeding programs while accounting for possible variance across various growth conditions and areas is highlighted by these findings.

3.2 Principal Component Analysis (PCA)

Deprived of a constant supply of genetic diverse plants, modern plant breeding would not be possible, much alone successful (Duvick, 2007). The initial stage in plant breeding is to determine genetic diversity in agro morphological capriciousness and characterization in plant breeding. As explained by Nelson (2011), plant breeders may need to gather germplasm from underutilized crops to generate improved cultivars that will feed fast-growing populations. Moradi et al. (2019); Dewi et al. (2020) identified as trait-assisted selection, trait variation could aids in the selection of the best lines for improvement.

The first three primary components of PCs 1 through 3 were generated from the mean data. PC1, PC2, and PC3 were maintained from the overall main components, accounting for 83.10% of the total variance, with values of 58.40%, 16.40, and 8.30%, respectively (Table 3 and Fig. 1). The components are ranked in order of preference using the Scree plot, which shows the relative distances between eigenvalues. Since their eigenvalues are greater than or equal to 1, the first three figures are considered important. The numbers then drop even more, and we conclude that these components are no longer as significant as they once were. As a result, we will exclude them from further studies. With eigenvalues of 8.76, 2.46, and 1.24, respectively,

Table 2. Pooled mean square of 15 agronomic, yield and related traits of 28 fenugreek accessions at Kulumsa in 2017 and 2018

Traits	Source of variations							
	Accessions (Df =27)	Year (Df=1)	Rep (Df =2)	Block (Rep) (Df =9)	Entry ×Year (Df=27)	Error (Df =90)	CV	R^2
DE	30.85***	96.01***	6.86	4.61	0.39^{ns}	0.97	9.77	0.92
DF	41.95***	41.01***	56.91	10.25	0.01***	2.45	3.07	0.86
DM	29.3***	0.02	6.93	2.84	0.02	3.19	1.54	0.75
GFP	21.98***	39.05**	83.80	17.00	0.03***	6.09	3.79	0.63
BY	14516887.9***	6171666.7***	111186.8	106236.5	102426.1*	216865.2	9.677	0.95
SY	1402197.07***	31673.03	5497.72	9223.89	2579.01	8785.99	6.75	0.98
TSW	17.63***	142.35***	0.13	0.90	0.0004***	2.20	9.89	0.76
PH	413.60***	234.68***	33.37	7.97	0.88***	13.81	8.299	0.90
PBPP	11.58***	47.39***	3.94	1.18	0.28	0.69	14.61	0.86
NFPDP	343.03***	691.58**	49.13	16.68	4.78	7.07	11.97	0.94
PL	7.62***	447.56***	1.55	2.91	0.12***	2.26	10.61	0.77
NSPD	5.27***	5.91*	0.07	2.01	0.06***	1.41	8.96	0.57
SYPP	15.04***	26.48***	0.86	0.21	0.10	0.41	14.67	0.92
NSPP	83454.38***	163797.59***	9226.22	3255.98	1009.43	2205.34	15.62	0.93
HI (%)	82.37***	104.85***	0.92	10.56	0.33***	8.75	10.19	0.76

*: p p≤0.05; **: p≤0.01; ns: non-significant difference; Df: Degrees of freedom; CV: Coefficient of variation; Rep: replication; DE: days to 50% emergence; DF: days to 50% flowering; DM: days to 50% physiological maturity; GFP: grain filling period; BY: biological yield (kg ha^{-1}); SY: seed yield (kg ha^{-1}); TSW: thousand seed weight (g); PH: plant height(cm) at maturity; PBPP: number of primary branches per plant; NFPDP: number of fertile pods per plant; PL: pod length per plant (cm); NSPD: number of seeds per pod; SYPP: seed yield per plant (gm); NSPP: number of seeds per plant; HI: harvest index (%)

PC1 showed 58.40% variability, followed by PC2 and PC2 with 16.40% and 8.30%, respectively, as seen in Fig. 1. In any further data analysis, these main component scores may be used to summarize the original 15 variables, which accounted for the majority of the variance (Table 3, Figs. 2 and 3). Similarly, Choudhary et al. (2022) reported that the first three principal components together accounted for 66.62% of the variation in a total of nine phenotypic variables among 48 genotypes of fenugreek, with eigenvalues greater than 1. Based on the number of seeds pod^{-1}, the number of pods plant^{-1}, number of primary and secondary branches, and seed yield (kg), the first, second, third, fourth, and fifth principal components accounted for 65.9% of the 160 fenugreek germplasm in Ethiopia, while the first and second principal components made up roughly 17.9% and 15.2% of the total variances (Roba and Mohamed, 2024). Fikreselassie et al. (2013) examined the degree of variability in morpho-physiological and nitrogen-fixing features across 143 Ethiopian fenugreek landraces and discovered that five of the ten PCAs explained more than 78.10% of the total variance. These findings are consistent with the current findings.

The first principal component (PC1) accounted for 58.40% of the total variation and was conquered by yield-related traits. Days to 50% flowering (0.30), thousand seed weight (0.28), number of primary branches plant^{-1} (−0.30), number of fertile pods plant^{-1} (−0.32), pod length (−0.28), number of seeds pod^{-1} (−0.28), seed yield plant^{-1} (−0.32), and number of seeds plant^{-1} (−0.32) were the morpho-agronomic traits that loaded most for the principal component (PC1). The second principal component (PC2) revealed 16.40% of the total variation, which related to traits such as days to 50% emergency (−0.39), plant height (0.5), grain filling period (−0.39), biological yield (−0.39), days to 50% physiological maturity (−0.38), and seed yield ha^{-1} (−0.32). The third principal component explained 8.30% of the total variation and was associated with the grain filling period (0.28), harvest index (−0.72), and thousand seed weight (0.32). The components and the variables exhibit both positive and negative correlation patterns, as indicated by the positive and negative loading. Positive and negative loading values were used to interpret component and variable correlations that were positive or negative. The cluster was more affected by the components with the highest absolute values in the first PC than by those with lower absolute values around zero (Chahal and Gosal, 2002). Table 3 and Fig. 2 shows the loading value, which illustrated the degree of association between the variables
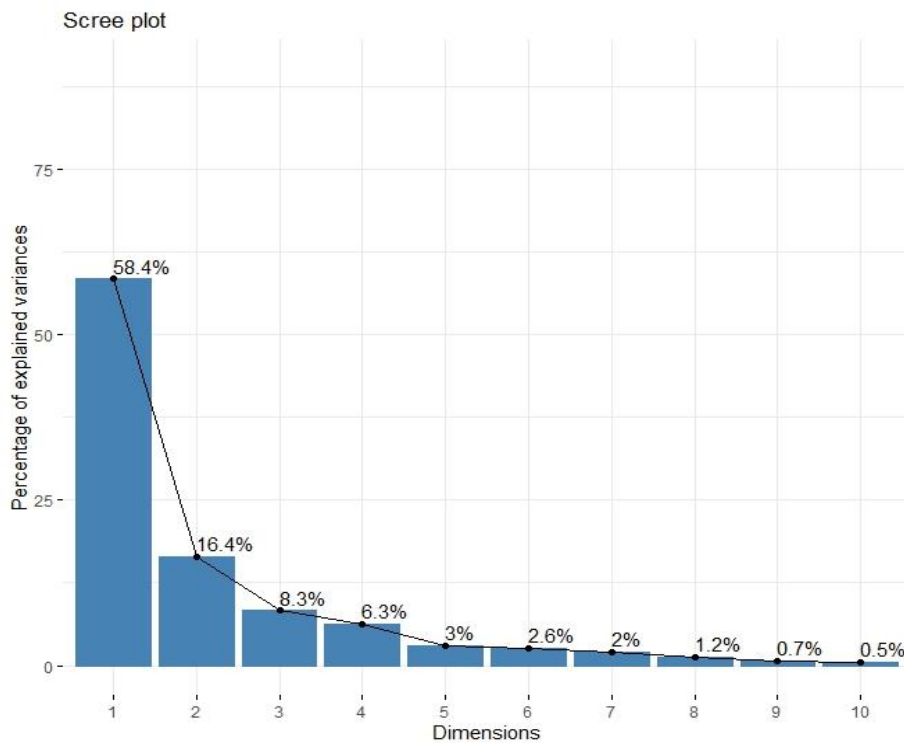

Fig. 1. The scree plot of the most important dimensions (pcs)

that were examined as well as the variance and correlations between them. According to Sneath and Sokal (1973), features with higher coefficients (eigenvectors) indicate their link to the pertinent PC axes.

As a result, the features listed above that load highly, either favorably or unfavorably, added most to the variety and were the ones that most set the clusters apart (Table 3, Figs. 2 and 3).

The features in the right two quadrants (Fig. 4) showed the most variability (variable PCA), and the accessions that are farthest from the origin (PCA Biplot) indicate the morpho-agronomic variety of the fenugreek accessions in comparison to those that are close to the origin

(Fig. 5). The higher contributors of accessions for the total variation were FG-53072, 201604, 219509, FG-20, FG-3, and 216900, respectively (Figs. 4 and 6) in the first two pcs. The lower contributors of accessions for the total variation were FG-8, 237984, and Chala.

3.3 Cluster Analysis

Based on standardized data from two seasons of mean values of 15 morpho-agronomic traits, the cluster analysis revealed 28 fenugreek genotypes that were typically grouped into three basic clusters based on the level of variation among the genotypes (Figs. 7 and 8 and Table 4). The mean values of the morpho-agronomic characters from each cluster are shown in Table 5.
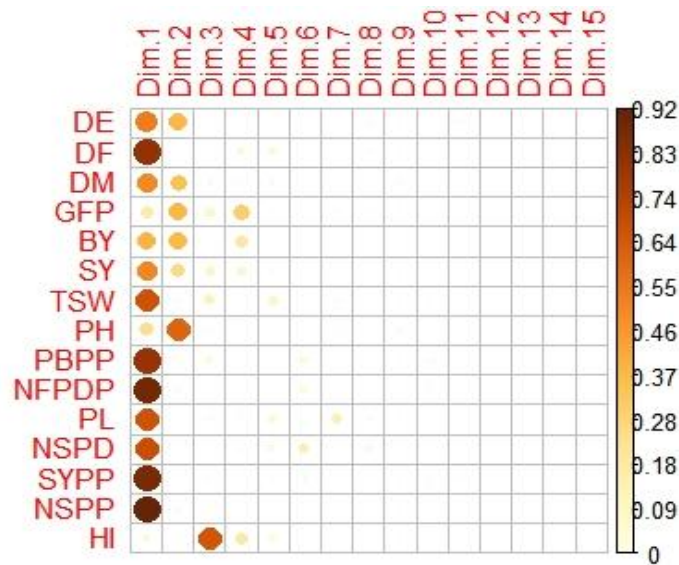

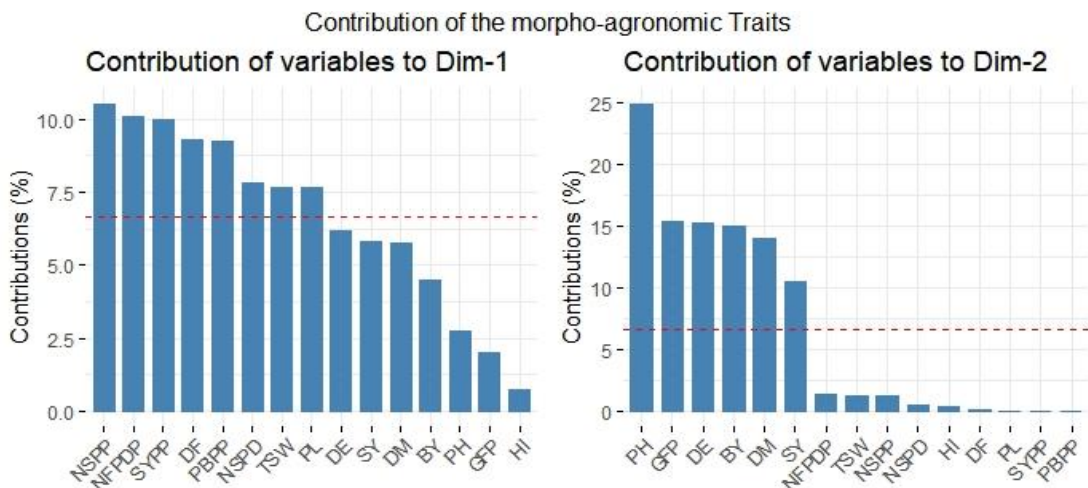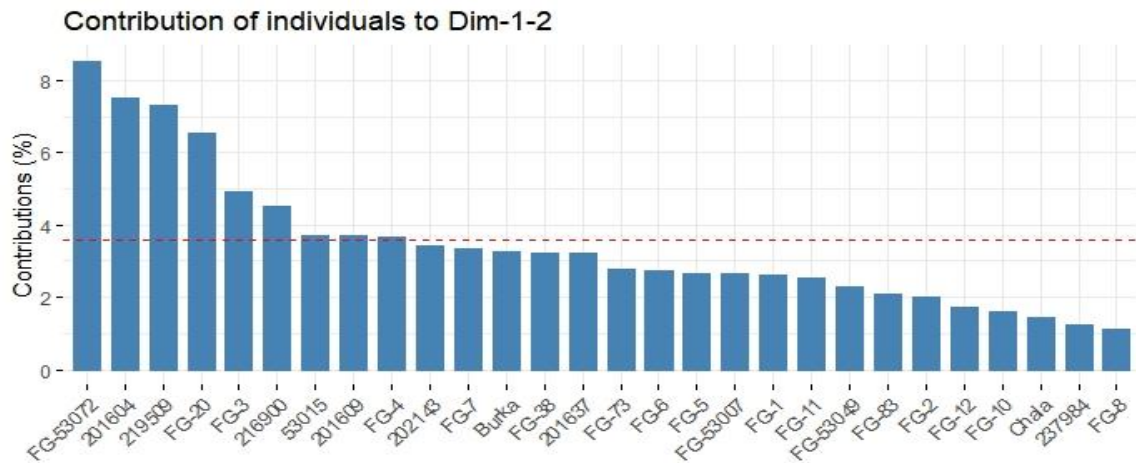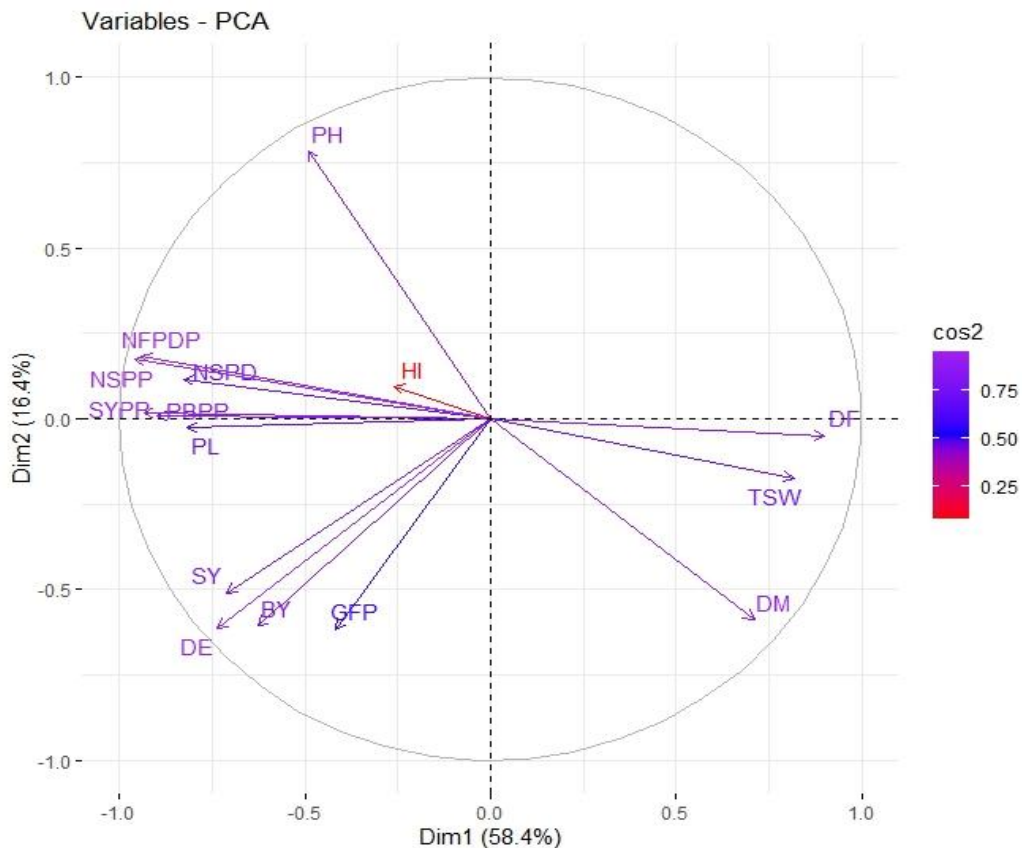**Fig. 2. The most contributing variables for each dimension**

Fig. 3. The highest contributors of the variables in the first two pcs

Fig. 4. Contributions of fenugreek accessions to the total variation to the first two PCS

Fig. 5. The variable PCA of 15 variables of 28 fenugreek accessions

The 28 fenugreek accessions were divided into three clusters based on morpho-agronomic traits by using the hierarchical agglomerative ward D^2 Dendrogram of the Euclidean distance clustering approach (Fig. 8). The number and names of accessions found in each cluster are shown in Table 4. Clusters I, II, and III included 11, 6, and 11 accessions out of a total of 28, which had

39.29%, 21.43%, and 39.29% of the accessions, respectively.

In agreement, Fikreselassie et al. (2013) reported five clusters for 143 studied land races collected from Ethiopia. Meena et al. (2021) stated the grouping of seventeen fenugreek genotypes in to six clusters, while Roba and

Mohammed (2024) outlined only two groups from 160 accessions collected from different parts of Ethiopia, which are not included in this study.

3.4 Mean Performance of Genotypes under Different Clusters

The cluster mean values for 15 traits are depicted in Table 5. The distinguishing characteristics of Cluster I are the highest mean values accessions for days to 50% emergency, days to 50% physiological maturity, days to grain filling period, number of primary branch plant⁻¹, pod length, and number of seeds pod⁻¹. This group of accessions has the smallest mean value for days to 50% flowering, seed yield ha⁻¹, and harvest index were primarily collected from different Oromia and SNNP.
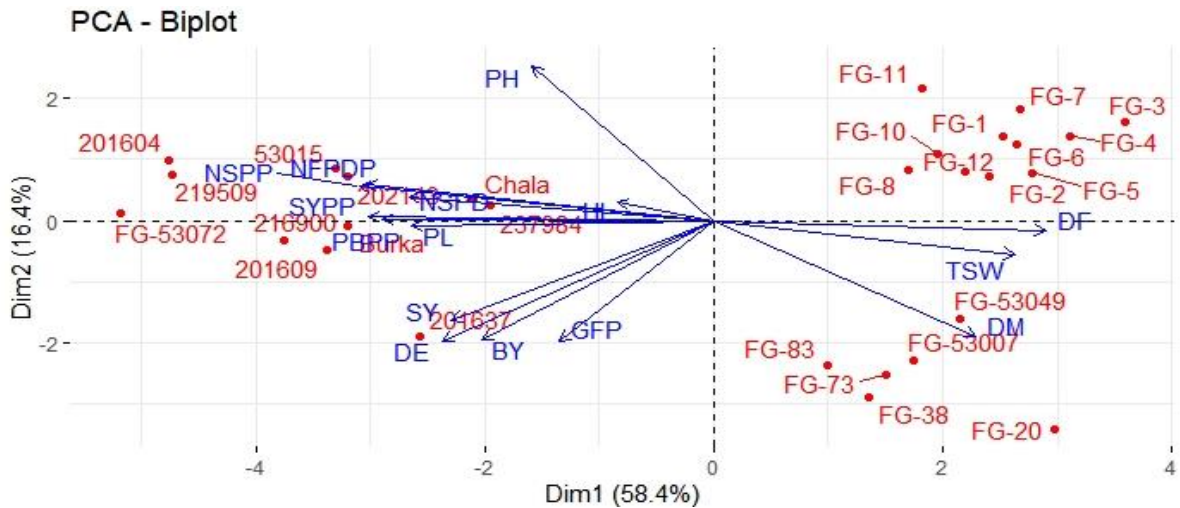

**Fig. 6. The PCA biplot (scatter plot) of both the morpho-agronomic traits and 28 fenugreek accessions**

Table 3. The first three principal components that explain the variation of 15 measured traits of 28 accessions

Traits	Eigen Vectors		
	PC1	**PC2**	**PC3**
DE	-0.25	**-0.39**	-0.10
DF	**0.30**	-0.03	-0.04
DM	0.24	**-0.38**	0.19
GFP	-0.14	**-0.39**	**0.28**
BY	-0.21	**-0.39**	-0.06
SY	-0.24	**-0.32**	-0.30
TSW	**0.28**	-0.11	**0.32**
PH	-0.17	**0.50**	0.16
PBPP	**-0.30**	0.01	0.22
NFPDP	**-0.32**	0.12	0.08
PL	**-0.28**	-0.02	0.19
NSPD	**-0.28**	0.07	0.08
SYPP	**-0.32**	0.01	0.13
NSPP	**-0.32**	0.11	0.08
HI	-0.09	0.06	-0.73
Eigen value	8.76	2.46	1.24
Variance percent	5.84	1.64	8.30
Cumulative variance	58.40	74.80	83.10

DE: days to 50% emergence; DF: days to 50% flowering; DM: days to 50% physiological maturity; GFP: grain filling period; BY: biological yield (kg ha⁻¹); SY: seed yield (kg ha⁻¹); TSW: thousand seed weight (g); PH: plant height(cm) at maturity; PBPP: number of primary branches per plant; NFPDP: number of fertile pods per plant; PL: pod length per plant (cm); NSPD: number of seeds per pod; SYPP: seed yield per plant (gm); NSPP: number of seeds per plant; HI: harvest index
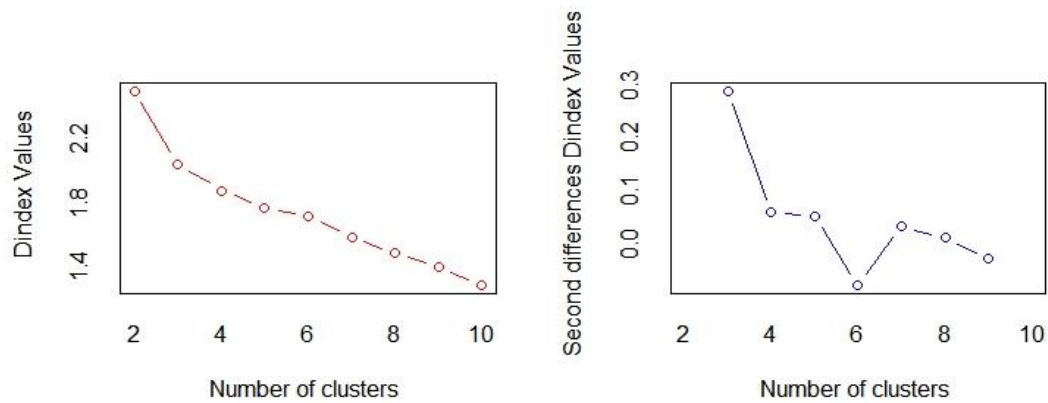
Fig. 7. The determined best number of clusters s in a data set according to the majority rule (viz Nbclust)

Cluster Dendrogram

d
hclust (*, "ward.D2")
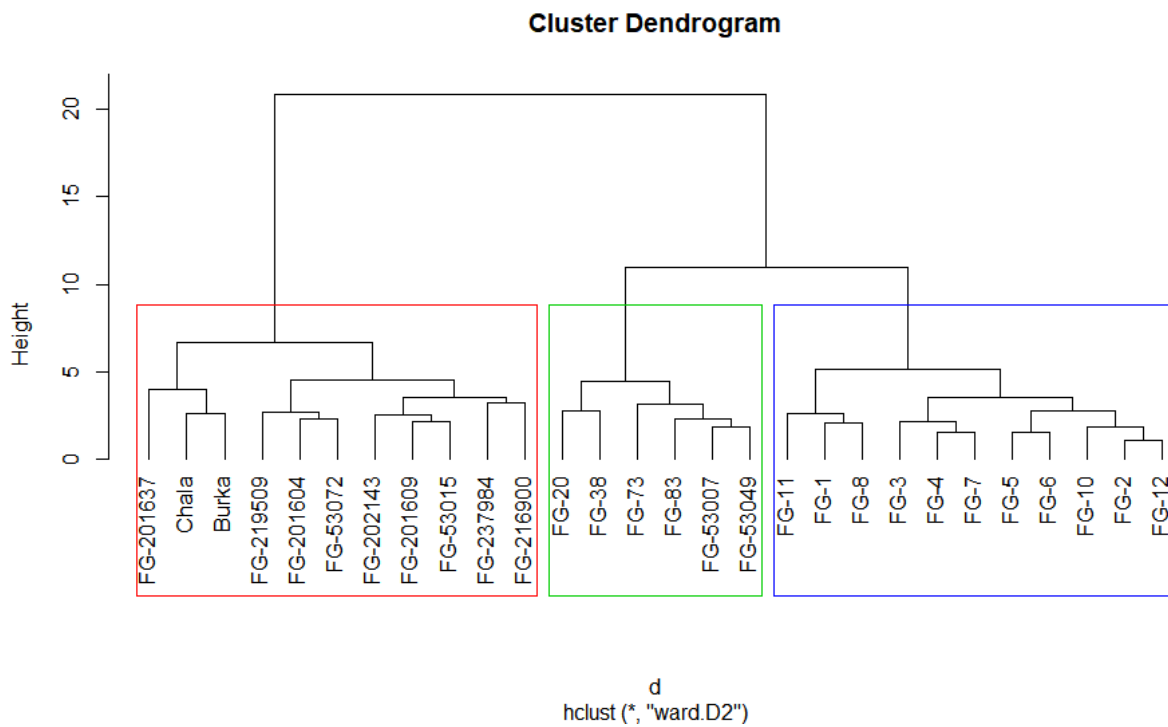
Fig. 8. Dendrogram showing genetic relationships among accessions using Ward's linkage method

Table 4. Grouping of 28 fenugreek accessions in to three different diversity classes

Clusters	Number of accessions	Percentage	Accessions
I	11	39.29	FG-2016637, Chala, Burka, FG-219509, FG-201604, FG-53072, FG-202143, FG-201609, FG-53015, FG-237984, FG-216900
II	6	21.43	FG-20, FG-38, FG-73, FG-83, FG-53007, FG-53049
III	11	39.29	FG-11, FG-1, FG-8, FG-3, FG-4, FG-7, FG-5, , FG-6, FG-10, FG-2, FG-12

The second cluster had the highest mean values of days to 50% emergency, days to 50% physiological maturity, and thousand seed weight.

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Table 5. Mean values of three clusters for 15 traits in 28 fenugreek accessions

Traits	Cluster		
	I	II	III
DE	11.98	7.36	11.56
DF	47.42	52.92	48.59
DM	111.58	117.37	113.33
GFP	68.17	64.45	64.74
BY	5129.85	4114.80	6342.27
SY	1397.07	1156.23	1947.07
TSW	13.87	16.08	12.95
PH	47.04	41.25	52.23
PBPP	7.48	4.73	6.99
NFPDP	27.94	16.79	32.14
PL	15.42	13.50	15.11
NSPD	14.21	12.69	14.18
SYPP	5.79	3.24	6.29
NSPP	397.06	214.34	455.31
HI	27.35	28.65	30.89

DE: days to 50% emergence; DF: days to 50% flowering; DM: days to 50% physiological maturity; GFP: grain filling period; BY: biological yield (kg ha^{-1}); SY: seed yield (kg ha^{-1}); TSW: thousand seed weight (g); PH: plant height(cm) at maturity; PBPP: number of primary branches per plant; NFPDP: number of fertile pods per plant; PL: pod length per plant (cm); NSPD: number of seeds per pod; SYPP: seed yield per plant (gm); NSPP: number of seeds per plant; HI: harvest index

The accessions included in this cluster are late maturing requiring extended period to mature and could be affected due to lack of moisture during their grain filling stages and resulted in shrinking of seeds and lower biological yield, seed yield ha^{-1}, plant height, number of primary branches, number of fertile pods, pod length, seeds pod^{-1}, seed yield plant^{-1}, and number of seeds plan^{-1}. Those genotypes could be utilized for areas with soil type, which stores moisture for long periods. Maximum mean seed yield ha^{-1}, plant height, biological yield, number of fertile pods plant^{-1}, seed yield plant^{-1}, and number of seeds plan^{-1} and harvest index were one of the common features of the accessions clustered in cluster III. These clusters could be targeted for further breeding programs to develop highyielding fenugreek varieties. This designates the opportunity of obtaining high-yielding segregating individuals through crossing with other clusters. The accessions from this cluster are very different from the first and the second cluster, signifying higher genetic differences. In accordance, Meena et al. (2021); Choudhary et al. (2022); Roba and Mohammed (2024) described the distinguishing feature for fenugreek genotypes or accessions as one cluster varies from the rest and selection depends on the merits of each cluster.

3.5 Intra and Inter-cluster Distances

Genetic progress through hybridization and selection be contingent on the level of inherent diversity between the respective parents based on the objectives and the merits of the groups of genotypes. The paired D^2 value was considered based on the combined mean values of the accessions.

The intra and inter-cluster distances of all clusters have been shown in (Table 6). The intra-cluster D^2 values ranged from 1.74 (cluster I) to 2.41 (cluster III). Within cluster distance was observed utmost in cluster III (2.41) followed by cluster II (1.94). However, the inter-cluster D^2 values varied between clusters I and II (5.92). The pairwise generalized squared distance (D^2)

Table 6. Intra (bold diagonal) distance and inter cluster (off-diagonal) distance analysis among 28 fenugreek accessions in three clusters (X^2 (0.05): 2.37; X^2 (0.01): 6.25)

	Clusters		
	I	II	III
I	**1.74**		
II	5.92*	**1.94**	
III	6.07*	3.94*	**2.41**

among the three clusters based on Mahalanobis's D^2 statistics revealed the maximum and highly significant genetic distance was observed between I and III (6.07), followed by clusters I and II (5.92), and the minimum inter-cluster distance observed between cluster I and III (3.94). As previously stated, the fundamental reason for diversity in a cluster's composition is large inter-cluster distance, which may be influences in a hybridization program. The most divergent clusters were observed between clusters I and III. On average, Cluster III is the most distant group among the three clusters. Maximum and minimum intra and inter cluster distances between fenugreek accessions or genotypes were also reported from previously by different scholars including Fikreselassie et al. (2013); Meena et al. (2021); Choudhary et al. (2022); Roba and Mohammed (2024). Those studies explained different magnitude of inter cluster distances and crossing between genotypes could produces high yielding progenies and those materials could be included in the breeding blocks.

The crosses involving parents from the most divergent groups will exhibit determined heterosis and higher variability in genomic architecture (Sing et al., 1987). In the present study, clusters with the highest inter-cluster distances were the most dissimilar. However, the chances of obtaining segregants with higher yields are quite limited when one of the clusters has a very low yield level (Samal et al., 1989), which is illustrated in cluster I. Therefore, in breeding programs, it is important to consider not only the genetic distance between clusters but also their mean performance for important agronomic traits in maximizing the chances of obtaining superior offspring. In accordance to the explained concepts in Chahal and Gosal (2002), the selection of parents for hybridization should also consider the specific advantages of each cluster and each accession within the cluster, depending on the specific goals of the breeding program. Therefore, based on the present results, it can be proposed that crosses involving cluster I with clusters II, and III exhibit high heterosis and may lead to segregation with respect to fenugreek accessions. The present study revealed significant morphological diversity among the tested accessions, suggesting opportunities to improve seed yield fenugreek through the crossbreeding of accessions from different clusters and subsequent selection from segregating and advanced generations. To increase the chance of producing high heterotic

hybrids and a broad spectrum of diversity in segregating generations, more genetically varied parents must be employed in the hybridization procedure (Desalegn et al., 2019; Wegayehu et al., 2023). Moreover, the most successful hybrids may include high-yield producing parents that have a wide range of genetic distinctions (Marker and Krupakar, 2009).

3.6 The Relative Contribution of Different Traits and Genotypes to Genetic Divergence

Through assessing the relative impacts of various traits on genetic differentiation, the effectiveness of the D^2 analysis is enhanced. Fig. 8 illustrates the agronomic factors contributing to diversity. The five most significant traits among all were plant height (25%), biological yield (15%), number of seed plant^{-1} (10.6 %), number of fertile pods plant^{-1} (10.00%), seed yield plant^{-1} (9.8%), days to 50% flowering (8.8%), and number of primary branch plant^{-1} (8.6%), which collectively represented the majority of the overall divergence. These traits were the primary contributors to the diversity among the evaluated accessions. In contrast, the divergence attributed to harvest index (0.5%) is minimal (Fig. 3). The most contributing genotypes to the genetic divergence were the following according to the aforementioned traits FG-53072, 201604, 219509, FG-20, FG-3, and 216900, respectively (Figs. 4 and 6). In accordance, Meena et al. (2021) reported 5 plants seed yield, plant height, and number of primary branches contributed maximum among the eight characters studied for genetic divergence between fenugreek genotypes at Ajmer.

4. CONCLUSION

The present result showed significant ($p \leq 0.01$) differences among fenugreek accessions for 15 studied traits. The number of seeds pod^{-1}, fertile pods plant^{-1}, seed yield plant^{-1}, days to 50% flowering, and plant height are the higher contributing traits to the difference between fenugreek accessions, accounting for 83.1% of the total variation from the first two pcs. Hybridization between those accessions included in I and II and I and III clusters could produce highly heterotic responses and segregants from the three clusters.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models

(ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study can be made available upon reasonable request.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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