



International Journal of
**Agricultural
Research**

ISSN 1816-4897



Academic
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Establishment of Distinct Core Collections based on the Predicted Genotypic Value of Ethiopian Fenugreek (*Trigonella foenum-graecum* L.) Landraces

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ABSTRACT

In order to evaluate selection procedures 144 random sample of fenugreek accessions collected from locations at heights ranging from 1750 to 3150 m, along with one released variety, were used in this study for designating a core collection representative of the whole range of accessions used in the study. The field experiment was conducted at Adadi and Ambo Research Center during 2006 and 2007 main cropping seasons. Treatments were arranged in a 12×12 simple lattice design. The data were subjected to multivariate analyses for different clustering methods, performed using the SAS software. Homogeneity test (F-test) for variance and a t-test for means ($p < 0.05$) were performed to determine the differences of traits between the core and the initial collections. Furthermore, the characteristic of the core collection in terms of the initial collection was determined by coincidence rate and the variable rate. Core collections established by distinct clustering and sampling methods were representatives of the initial collection, with exception of the collection constructed by Ward's clustering method combined with random sampling. This observation indicates that the genetic variation available in the initial collection can be preserved in the nine established core collections. These core subsets can be used as a point of entry for efficient and effective exploitation of germplasm resources for the improvement of the crop.

Key words: Core collection, *Trigonella foenum-graecum* L., stepwise cluster, sampling strategy, genotypic value

INTRODUCTION

Fenugreek is belongs to the genus *Trigonella* which is the largest genera of the family Fabaceae (Balodi and Rao, 1991). The genus consists of approximately 70 species that are annuals and native to Southern Europe (Westphal, 1974; Jami and Odurudwe, 1987; Engles *et al.*, 1991). *Trigonella foenum-graecum* (commonly known as fenugreek) is an annual species with hermaphrodite flower. Since its petal color is white, the crop fenugreek is occasionally visited by insects.

Core collections are grouped based up on the hierarchical structure of the gene pool in the entire population. The entire collection (population) can be stratified into smaller groups which shares common features based up on taxonomy, geographic or ecological origin and neutral or non-neutral descriptors, followed by sampling within these groups (Huaman *et al.*, 1999; Ortiz *et al.*, 1998). Using this stratification process core subsets can be identified. Genetic studies in different cultivated

crops indicated that common widespread and localized alleles in the entire collection are contained in the established core subset (Huaman *et al.*, 2000). Ortiz *et al.* (1999), the core subset provides information to the entire collection for further investigation of biodiversity or direct utilization of these resources. In addition, studies on diversity, documentation, conduction of seed multiplication and periodic rejuvenation, or establish *ex situ* conservation in cold rooms are costly, time-consuming and tedious. The core collection concept was proposed to overcome the difficulties by reducing a large collection to a more easily and cost-effectively manageable size. The variability of the smaller collection, however, is representative of the entire collection. In line with this, some gene banks have large germplasm collections, which often ineffectively managed and not widely accessed by the breeders. The investigation of the genetic variability in the entire genetic resources has helped to establish a workable collection. Core collections constitute subsets of large germplasm collections, containing chosen accessions that capture most of the genetic variability in the entire collection. Therefore, establishing a core collection can improve the efficiency of managing and utilizing a germplasm collection.

Dahlberg *et al.* (2004), suggested that the core should provide a road-map from which breeders can describe genetic diversity of crop plants and also further improves the competences of isolating and cloning genes of interest in the future. According to Frankel (1984) and Frankel and Brown (1984), a core collection is a sub sample of a larger germplasm collection that contains, with a minimum number of repetitiveness, the maximum possible genetic diversity of the species in question. It means that a core collection is a limited set of accessions of a crop species and its wild relatives, chosen to represent all or most of the genetic diversity held in the larger collection. Core collections can help to increase the use of germplasm collections, some of which may contain many thousand accessions of a single species. Often, plant breeders may not sufficiently draw on materials housed in large collections, due to the cost and impracticality of carrying out in depth studies on so much material. A core collection can be established to identify useful genes for use by germplasm curators, plant breeders, farmers, researchers and others.

Various core collections have been established for distinct crops around the world (Li *et al.*, 2004). According to the same authors, the selection of an appropriate sampling strategy and a clustering method are important in the construction of core collections based on predicted genotypic values in order to retain the greatest degree of genetic diversity from the initial collection. The authors also ascertain that core collections sampled using predicted genotypic values encompassed more genetic diversity than those based on phenotypic values.

Genetic sorting based on phenotypic data may not correctly reflect the genetic diversity of initial germplasm resources. If genotypic values can be predicted based on phenotypic values, the genetic distance, based on genotypic value among accessions, can be more accurately measured. A core collection constructed by genotypic values will be more representative of the initial collection (Hu *et al.*, 2000a).

Further neglect and under-use of the locally important crop fenugreek will inadvertently entail the risk of loss of important germplasm material developed over thousands of years of cultivation. However, one important factor restricting its large-scale production and the development of better varieties is the low volume of available information on the species genetic diversity. Therefore, in view of filling up such a technical gap, the current research was conceived to establish a fenugreek core-collection based on 144 germplasm.

MATERIALS AND METHODS

The field experiment was conducted at two locations at Ambo and Adadi, during 2006 and 2007 main cropping seasons. Ambo is located at an altitude of 2300 m above sea level (m.a.s.l.) and is

submitted to an average annual rainfall of 1000 mm, while Adadi is located at an altitude of 2050 m.a.s.l. and displays an average annual rainfall of 900 mm (Gemechu, 2012). The soil at Ambo is classified as vertisol with a pH value of 6.1, while Adadi soil is classified as a light vertisol with pH 7.5.

One hundred forty-three random samples of fenugreek accessions, along with one commercial variety (*Challa*), were employed in this study. The accessions were collected by the Institute of Biodiversity Conservation (IBC) from the most important production complexes of Ethiopia, representing distinct agroecological contexts of varying altitude, rainfall, temperature and soil type (Million, 2012).

Treatments were arranged in a 12×12 simple lattice design. Seeding was carried out in a plot of four rows 2 m in length and regular spacing of 10 cm between plants and 25 cm between rows. The layout and randomization were as per the standard procedure set by Cochran and Cox (1957). Two seeds per hole were carefully placed to ensure stand germination. Thinning was performed at the true-leaf stage. Weeding and other cultural practices were done as per the recommendations adopted for the respective sites.

The following data were collected during 2006 and 2007, either from whole plot or from ten randomly sampled plants from each plot. Mean values of the samples were utilized to estimate the performance of each germplasm accession for the investigated traits. Detailed accounts of each data type and collection methods are discussed hereunder.

Days to flowering: The number of days, from the date of sowing to the date at which 50% of the flower buds of the plants in a plot had blooms.

Thousand seed weight: Weight of 1000 seeds, in grams, at 12% moisture content, determined from the average weight of two samples of 250 seeds multiplied by four.

Number of pods per plant: The average number of pods counted from ten sampled plants before harvesting.

Plant height: Average height, measured in centimeters, from the ground level to the tip of the last pod on the main stem of the plants used to determine number of pods per plant. It was determined at the date of harvest.

Number of seeds per plant: The average number of seeds per individual plant obtained from the plants used to determine the number of pods per plant.

Number of seeds per pod: Average of the total number of seeds in the pods from the ten sampled plants, rounded to the next whole number.

Seed yield/plant: The average weight, in gram, of seeds adjusted 12% moisture content obtained from the ten sampled plants.

Biomass yield per plot and per plant: The aerial-part biomass yield, in grams per plot and per plant, determined from the net plot size and the ten sampled plants at the time of maturity, respectively. Genetic analysis was performed on plant-basis.

Harvest index (HI): It was recorded as the ratio of the moisture-adjusted seed yield per plant to the aerial biomass yield per plant as given by the formula:

$$\text{HI}(\%) = \frac{\text{Moisture - Adjusted seed yield (g plant}^{-1}\text{)}}{\text{Biomass yield (g plant}^{-1}\text{)}} \times 100$$

Days to maturity: Recorded as the number of days from sowing to the date at which 90% of the pods reached the stage of physiological maturity.

Grain filling period: Recorded as the number of days from “days to flowering” to “days to maturity”.

Seed protein content: Representative bulk seed samples from randomly chosen fifty plots were oven-dried to constant moisture, ground to pass through a 2 mm size mesh sieve for determination of nitrogen content at Holetta Research Center using the Kjeldahl technique. Grain protein contents were estimated by multiplying percentage of N in the dried seed samples by the standard conversion factor of 6.25 (AOAC, 1970). The results obtained from the fifty sampled plots were used for calibration and validation of Near Infrared Reflectance Spectroscopy (NIRS), which is also available at Holetta Research Center. Thus, the remaining experimental material was analyzed by taking 3 g of intact seeds from each plot and scanning using a monochromator model 6500, NIR systems.

Number of nodes per plant: Determined as an average number of nodes from the ten sampled plants.

Number of Podding nodes per plant: Determined as an average number of nodes bearing pods from the ten sampled plants.

Number of primary branches per plant: The total number of primary branches that gave rise to other seed-bearing branches of higher order or born seeds themselves was determined as an average number of productive primary branches from the ten sampled plants. Counting was performed at the time when flowering was completely over and pods were still green but old enough to visually evaluate seed production.

Number of secondary branches per plant: The average number of secondary or any other higher order productive branches of the plants used to determine the number of primary branches.

Number of nodules/plant: The average number of nodules obtained from the five sampled plants. The results were scored using a 0-4 scale, where 0 = no nodule, 1 = 1 to 10 nodules/plant; 2 = 11 to 20 nodules/plant; 3 = 21 to 30 nodules/plant and 4 = more than 30 nodules/plant.

Fresh weight of nodules/plant: The average weight, in gram per plant, of fresh nodules obtained from the plants used to determine number of nodules per plant.

Number of effective nodules/plant: The number of effective nodules was visually identified at the time of flowering from the plants used to determine “number of nodules per plant” and “fresh

weight of nodules per plant". The effective nodules were differentiated from ineffective ones by their color, whereby the deep red- or brown-colored nodules, caused by which reflect high leghaemoglobin content, were designated as effective, while others were designated as non-effective nodules (Carter, 1995).

Core collection was constructed by stepwise clustering with three sampling strategies based on genotypic values, as described by Hu *et al.* (2000b). The genotypic values were determined using the linear mixed model of Zhu and Weir (1996) for analyses of genotype \times environment interactions as used by Alemayehu and Becker (2002), representing the following model:

$$P_{(v)} = \mu + E_h + G_i + GE_{ih} + B_{k(h)} + e_{hg(i)}$$

where, $P_{(v)}$ = phenotypic value, μ = grand mean, E_h = effect of the hth environment, G_i = genotypic effect of the ith accessions, GE_{ih} = interaction between the ith accession and the hth environment, $B_{k(h)}$ = effect of block k in hth environment and $e_{hg(i)}$ = residual (random error).

Mahalanobis distance (Mahalanobis, 1936) was calculated by a variance-covariance matrix in order to investigate the correlations among traits and eliminate the scalar differences between the traits. For accession grouping, the hierarchical cluster analyses based on unweighted pair-group average method (Sokal and Michener, 1958), Ward's method (Ward Jr., 1963) and the complete linkage method (Sorensen, 1948) were performed using the SAS software (SAS, 1996). Based on the dendrogram of clusters, three sampling strategies, combined with stepwise clustering, were developed. The characteristics of each clustering and sampling strategy are illustrated below.

Unweighted pair-group average: In this method, the distance between two clusters is calculated as the average distance between all pairs of objects in two different clusters. This method is also very efficient when the objects form natural distinct "clumps," however, it performs equally well with elongated, "chain"-type clusters. Note that in their book, Sneath and Sokal (1973) introduced the abbreviation UPGMA to refer to this method as unweighted pair-group method using arithmetic averages.

Complete linkage (furthest neighbor): In this method, the distances between clusters are determined by the greatest distance between any two objects in different clusters (i.e., by the "furthest neighbors"). This method usually performs quite well in cases when the objects actually form naturally distinct "clumps." If the clusters tend to be somewhat elongated or of a "chain"-type nature, then this method is inappropriate.

Ward's method: distinct from the other methods due to the use of an analysis of variance approach to evaluate the distances between clusters. In short, this method attempts to minimize the Sum of Squares (SS) of any two (hypothetical) clusters that can be formed at each step as described by Ward Jr. (1963). In general, this method is regarded as very efficient; however, it tends to create clusters of small size.

Random sampling: For this strategy, from each respective cluster, one accession from each subgroup either two accessions at the lowest level of sorting were randomly selected. If there is only one accession in a sub-group, it was directly sampled for the next cluster.

Preferred sampling: For this strategy, accessions with maximum or minimum values of traits were preferred for selection from each subgroup of the respective clustering methods, at the lowest

level of sorting. Both accessions are selected if two accessions in a subgroup have maximum or minimum values of the traits. The other procedures are similar to the random sampling strategy.

Deviation sampling: The degrees of deviation of two accessions were compared in each subgroup, at the lowest level of sorting; the accession with larger degree of deviation was selected for the next accession. The accession with larger degree of deviation is selected for the subsequent cluster analysis. If there is a single accession in a subgroup, it is also directly sampled to the next cluster.

The genetic distance among all accessions selected based on the dendrograms from the first cluster analysis were calculated. Then, the second cluster analysis of the accessions was performed and accessions were selected based on a new dendrogram of clusters by one of the three sampling strategies. Similarly, the stepwise cluster analyses were conducted until the number of selected accessions was reduced to 20-30% of the number of accession in the initial collections (Crossa *et al.*, 1995; Yonezawa *et al.*, 1995).

Evaluation of the core collection: Homogeneity test (F-test) for variance and a t-test for means ($p < 0.05$) were performed to determine the differences of traits between the core and the initial collection. The percentage of significant difference between the core and the initial collection was calculated for the mean different percentage (MD%) or the variance percentage (VD%) of traits (Hu *et al.*, 2000b). Core subsets displaying low (near to zero) MD% and wider or larger VD% values were considered better representatives of the initial collection.

The property of the core collection in terms of the initial collection was determined by the coincidence rate (CR%) and the variable rate (VR%), as suggested by Hu *et al.* (2000b). The core collection is considered to be the representatives of the initial collection according to the following conditions:

- No more than 20% of the traits have distinct means (significant at $\alpha = 0.05$) between the core and the initial collection (144 accessions in the current study) and
- Retained CR% in the core collection is not smaller than 80%

RESULTS AND DISCUSSION

As pointed out earlier, this investigation was conducted to establish a representative core-collection for a whole range of 144 accessions used in the current study.

The procedure used to select the core collection from the 144 fenugreek accessions obtained from IBC of Ethiopia resulted in nine possible core collections, which were developed using three clustering methods (unweighted pair-group average linkage method, C1; complete linkage method, C2; and Ward's method, C3) combined with three sampling strategies (deviation sampling, S1; preferred sampling, S2; and random sampling, S3). The nine obtained core subsets were named as CorC1S1, CorC1S2, CorC1S3, CorC2S1, CorC2S2, CorC2S3, CorC3S1, CorC3S2 and CorC3S3.

There were no significant differences ($p < 0.05$) in the means of all traits, between each of the nine core collections and the initial collection. The value for MD% was 0 and CR% was larger than 80% for the nine collections, with exception of CorC3S3 (Table 1). This indicates that all developed collections, except for CorC3S3, obtained using distinct clustering and sampling methods, were representative of the initial collection.

The collections CorC1S1, CorC2S1 and CorC3S1 were developed by deviation sampling combined with the unweighted pair-group average method, complete linkage method and Ward's method, respectively. In comparison to both, the complete linkage and Ward's methods (Ward Jr., 1963), the unweighted pair-group average method exhibited a trend of providing medium VD%

Table 1: Percentage of trait differences between the developed core collections and the initial collection of Ethiopian *T. foenum-graecum*

Statistic (%)	CorC1S1	CorC1S2	CorC1S3	CorC2S1	CorC2S2	CorC2S3	CorC3S1	CorC3S2	CorC3S3
VD ^a	15.56	66.77	33.28	14.07	52.65	40.52	16.27	51.87	16.63
MD ^a	0.00	0.00	0.00	0.0	0.00	0.00	0.00	0.00	0.00
CR	81.70	91.77	82.12	83.26	93.94	87.40	81.90	82.27	74.26
VR	97.68	121.82	106.17	96.76	118.66	109.86	101.82	112.95	95.77

^aPercentage of significant difference (p = 0.05) between core collection and the initial collection for variances (VD) and means (MD) of traits, CR: Coincidence rate, VR: Variable rate

values, low and similar CR% in comparison to Ward’s method and medium and similar VR% in comparison to the complete linkage method. Complete linkage method gave high CR% but low VR% and VD% as compared to both clustering methods. Considering Ward’s method, low CR% but higher VR% and VD% were obtained (Table 1). Therefore, considering all investigated parameters, it can be concluded that complete and ward’s method were slightly better than the unweighted par-group average method, when deviation sampling was used to develop the core collections.

Three core collections were developed by preferred sampling combined with unweighted pair-group average (CorC1S2), complete linkage (CorC2S2) and Ward’s (CorC3S2) methods.

The unweighted pair-group average resulted in the highest VD and VR% in comparison to both clustering methods, although the method produced intermediate CR%. The complete linkage method resulted in the highest CR%, but intermediate VD and VR% values. In a similar fashion, the use of Ward’s method generated lower VD, CR and VR%, in comparison to both clustering methods. From the result, it can be concluded that the complete linkage and unweighted pair-group average methods were better than the Ward’s method when preferred sampling strategy was used to develop the core collections. This result was the similar to those obtained by Hu *et al.* (2000a), who demonstrated the construction of different core collection by using 21 traits on 168 cotton germplasm accessions.

When a random sampling strategy was considered, three core collections were obtained by combining the unweighted pair-group average (CorC1S3), complete linkage (CorC2S3) and Ward’s (CorC3S3) methods. The use of the complete linkage method gave higher VD, CR and VR%, followed by intermediate values resultant from unweighted pair-group average clustering in comparison to Ward’s method. Based the results, it can be concluded that the complete linkage method was better than the remaining tested clustering methods and the unweighted pair-group average was better than the Ward’s method, when the random sampling strategy was used to construct the core collection.

Based on the results obtained, from the three clustering methods investigated, a better representation of the initial collection was obtained in CorC2S1 by deviation sampling, in CorC1S2 and CorC2S2 by preferred sampling and in CorC2S3 by random sampling. These observations indicate that the use of distinct clustering methods on a given dataset will provide different results. The basic similarity of all clustering methods is that they use some similarity (proximity) or dissimilarity (distance) measures to classify the objects into groups.

The effectiveness of the different sampling strategies for core collections was compared under the conditions of a single cluster method. No significant differences were observed for the trait means between the initial collection and the core collections developed by the three sampling strategies (Table 1).

Three core collections were constructed by the unweighted pair-group average method combined with deviation (CorC1S1), preferred (CorC1S2) and random (CorC1S3) sampling strategies. When

using the preferred sampling strategy, CorC1S2, the highest VD, CR and VR% were observed, with intermediate values obtained from random sampling. However, the use of a deviation sampling strategy gave the lowest VD, CR and VR%, when the unweighted pair-group average method was considered (Table 1).

Three core collections were developed by complete linkage method with deviation (CorC2S1), preferred (CorC2S2) and random (CorC2S3) sampling techniques. Among the three core collections, the one constructed by preferred sampling technique gave the highest VD, CR and VR%. Random sampling strategy gave intermediate values of VD, CR and VR%, whereas deviation sampling gave the lowest CR and VR% (Table 1). Among the three core collections developed by the Ward's method combined with deviation (CorC3S1), preferred (CorC3S2) and random (CorC3S3) sampling techniques, the core collection established by preferred sampling strategy, CorC3S2, gave the highest VD, CR and VR% (Table 1).

Therefore, the properties of core collections developed by the three sampling strategies were similar when using the unweighted pair-group average and complete linkage methods and were nearly equivalent when using the Ward's method. It was evident that the properties of the core collections developed by all three sampling strategies were stable and the collections, feasible. Similar results were observed by Hu *et al.* (2000a).

Differences among means of the initial collection and the nine established core collections are presented in Table 2. The result revealed that among the nine-core subsets, only the core collections established by the deviation sampling technique gave significant mean differences in some traits, between the core and initial collections.

Table 2: Mean values for 19 traits recorded in the initial collection and nine core subsets of *T. foenum-graecum* accessions

Variables	Initial collection	CorC1S1	CorC1S2	CorC1S3	CorC2S1	CorC2S2	CorC2S3	CorC3S1	CorC3S2	CorC3S3
DF	50.86	51.540**	50.69	51.18	51.49*	50.67	50.73	51.40*	50.94	50.74
PH	33.56	34.350	33.44	33.12	34.67	33.61	33.57	34.03	33.29	34.12
BMY	7.26	7.630	7.30	7.25	7.65	7.27	7.46	7.59	7.37	7.35
PPP	15.56	16.900	15.72	15.91	16.92	15.63	15.74	16.03	16.08	16.28
SPPL	166.85	168.370	165.08	167.58	171.72	166.23	169.90	167.34	165.33	172.27
SPP	10.67	9.860	10.43	10.47	10.06	10.59	10.63	10.25	10.36	10.65
TSW	17.19	17.850	17.54	17.12	17.82	17.15	17.45	17.86	17.17	17.15
SY	2.86	3.030	2.88	2.86	3.08	2.85	2.97	3.02	2.84	2.93
HI	38.86	38.750	38.74	38.99	38.96	38.86	39.30	38.91	38.48	38.67
DM	116.46	117.750*	116.33	116.41	117.82*	116.44	116.58	117.26	116.84	116.48
GFP	75.57	76.640	75.44	75.20	76.45	75.59	75.79	76.18	75.49	75.69
NPPL	29.20	31.090	30.32	29.47	31.51*	30.02	29.47	30.66	30.08	30.95
PNPPL	9.61	10.650	10.13	9.82	10.61	9.94	9.67	10.65	10.15	10.19
PBR	3.08	3.240	2.89	3.18	3.19	2.90	2.98	3.25	3.09	3.16
SBR	0.43	0.580	0.61	0.49	0.51	0.53	0.54	0.45	0.47	0.53
NOPPL	11.55	11.810	12.28	12.66	11.63	12.07	13.14	11.36	11.72	12.14
ENOPPL	2.16	2.230	2.13	2.32	2.09	2.02	2.40	2.23	1.85	2.39
FWNOPPL	0.13	0.130	0.13	0.14	0.12	0.13	0.13	0.13	0.13	0.12
CP%	27.83	28.110	27.58	28.00	28.22	27.66	27.91	28.40*	27.81	27.96

DF: Days to 50% flowering, PH: Plant height (cm), BMY: Biomass yield (g per plant), PPP: No. of pods per plant, SPPL: No. of seeds per plant, SPP: No. of seeds per pod, TSW: Thousand seeds weight (g), SY: Seed yield (g per plant), HI: Harvest index in percentage, DM: Days to 90% maturity, GFP: Grain filling period, NPPL: No. of nods per plant, PNPPL: No. of podding nods per plant, PBR: No. of primary branch per plant, SBR: No. of secondary branch per plant, NOPPL: No. of nodule per plant, ENOPPL: No. of effective nodules per plant, FWNOPPL: Fresh weight of nodules (g per plant) and CP: Crude protein of the seed, ***:Significant at 0.05 and 0.01 probability level, respectively

From the 19 studied characters in the core collections established by the unweighted pair-group average method with the deviation sampling technique (CorC1S1), only the mean of two traits were statistically different from that of the initial collection. From these characters, days to flowering were highly significant and days to maturity were significant (Table 2). With the complete linkage method combined with deviation sampling technique (CorC2S1), the mean of three characters was statistically distinct from that of the initial collection: days to flowering, days to maturity and number of nodes per plant were significantly different from the initial collection (Table 2).

The means of two traits from the core collection established by the Ward's method combined with deviation sampling strategy (CorC3S1) were significantly different from those of the initial collection: days to flowering and crude protein content of the seed showed significantly differed from the initial collection (Table 2).

CONCLUSION

Better representation of the initial collection was obtained using complete linkage method, among the three clustering methods tested for establishing a core-collection,. Therefore, the properties of core collections developed by the three sampling strategies were the equivalent when using unweighted pair-group average and complete linkage methods. The properties were nearly equivalent when using the Ward's method. It was evident that the properties of the core collections developed by all three sampling strategies were stable and the subsets were feasible in which they represented the entire collection. Among the nine core subsets, only the core collections established using the deviation sampling technique gave significant mean differences, for some traits, in comparison to the entire collections.

Finally, a collection of 144 fenugreek accessions, representing approximately 38% of the country's fenugreek germplasm collection, was evaluated. The resources available for germplasm evaluation are limited and declining steadily. Therefore, extensive evaluations of the entire germplasm collection are not possible. The established core subsets, developed in the current study, will be used as working fenugreek germplasm collection, allowing its extensive survey for economically important traits, in future breeding programs. Further genotyping studies may help to provide suitable ways of deciding the number of accessions selected from each group.

ACKNOWLEDGMENTS

This report is part of M.Sc. research thesis work of the first author. The authors wish to thank all the staff of the Highland Pulse Research Program at Holetta Research Center in general and Dr. Gemechu Keneni and Mr. Mussa Jarsso in particular for their immense assistance in the research work are highly acknowledged.

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