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Multivariate Analysis of Genetic Divergence among Ethiopian Mustard (*Brassica carinata* A. Brun) Genotypes in Relation to Seed Oil Quality Traits

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ABSTRACT

A study was conducted to assess the extent and pattern of genetic variability of Ethiopian mustard genotypes with respect to eight seed oil quality traits in 36 Ethiopian mustard genotypes at Holetta Agricultural Research Center, Ethiopia. The experiment was laid out in simple lattice design. Univariate analysis of variance has shown that there was significant variation among genotypes in all traits. Multivariate analysis has resulted in the formation of seven clusters and has shown the presence of substantial genetic diversity for further selection and breeding. Genetic distances among most clusters were significant from which selection of parents may be made for crossing in order to obtain genetic recombination and transgressive segregants. Genotype in cluster 7 was relatively the highest in oleic and the lowest in erucic acid in its seed oil. Likewise, genotypes in cluster 2 and 4 showed the highest erucic acid and the highest oil content in their seeds, respectively. Apart from selecting genotypes from the clusters which have higher inter-cluster distance, within a cluster performance of genotypes should also be considered for a particular trait of interest. The cumulative effects of individual traits were responsible for differential grouping of genotypes. The present investigation also revealed that diverse geographic region, though important, it could not be the only index of genetic variations, in which selection pressure, environment and genetic drift may also be the cause.

Key words: Ethiopian mustard, genetic diversity, multivariate analysis, quality traits, univariate analysis

INTRODUCTION

Ethiopian mustard (*B. carinata*) is one of the six economically important *Brassica* species which is believed to be originated in the highlands of the Ethiopian plateau and the adjoining portion of East Africa and the Mediterranean coast (Gomez-Campo and Prakash, 1999). It evolved as a natural cross between *B. nigra* (BB) (n = 8) and *B. oleracea* (CC) (n = 9) and under went further chromosomal doubling (2n = 34; Nagahari, 1935).

In Ethiopia, it is cultivated as an oilseed crop since ancient time and third in its production next to noug (*Guizotia abyssinica* Casa) and Linseed (*Linum usitatissimum* L.) (Alemayehu and Becker, 2001). Though, it is the highest in productivity, high erucic acid content in the seed oil, making it a poor choice for use of seed oil for food, the oil is very often adulterated with premium oil from

noug (Alemayehu and Becker, 2002). There are opportunities which favor cultivation of oilseeds in general in the country which ranges from import substitution of edible oils to export of high value seed and oil. Increasing the supply of oils and fats is imperative where its per capita availability is quite low like Ethiopia. On the other hand, the oil shows physical and chemical properties suitable for bio-diesel (Cardone *et al.*, 2003) which can also substantially contribute to the economic development of the country.

Therefore, in order to use Ethiopian mustard seed oil for food and non food industry and enhance its cultivation, different seed oil quality characteristics have to be fulfilled. There are various breeding strategies which enable the improvement of oil quality demanded by the aforementioned market segments (Alemayehu and Becker, 2001; Getinet *et al.*, 1994; Alonso *et al.*, 1991; Fernandez-Escobar *et al.*, 1988; Velasco *et al.*, 1995; Teklewold, 2005).

Although efforts have been done to improve oil quality of Ethiopian mustard seed using various breeding strategies, much need to be done to utilize natural variations that might exist among population of the species for fatty acid profile. Doing so, may help for future breeding procedure such as backcrossing and development of agronomically viable genotype. Assessing the genetic diversity and relationship among Ethiopian mustard genotypes based on their seed oil quality traits is thus a prerequisite which may help in identifying important genotypes and selection criteria for improvement of Ethiopian mustard seed oil. This study was, therefore, executed with the objective of assessing the extent and pattern of genetic variability of Ethiopian mustard genotypes of diverse agro climatic regions of the country.

MATERIALS AND METHODS

The experiment was conducted at Holetta (38°E and 9°N) Agricultural Research Center (HARC) in 2010 cropping season. Holetta is one of the representatives of oil seed *Brassica* growing areas in the central highlands of Ethiopia (Alemayehu and Mesfin, 1994) with its annual rainfall of 1100 mm, altitude of 2400 m a.s.l and temperatures of 22°C (maximum) and 6°C (minimum). Thirty six genotypes of Ethiopian mustard including the standard checks were used in the study. The genotypes were collected by formerly Institute of Plant Genetic Resource Conservation and Research, currently Institute of Biodiversity Conservation (IBC) from diverse geographical region of the country. Genotypes by origin are described in Table 1. The experiment was carried out using

Table 1: List of genotypes considered in the study and their origin

Code	Acc. No	Area of collection	Altitude (m)	Code	Acc. No	Area of collection	Altitude (m)	Code	Acc. No	Area of collection	Altitude (m)
1	20052	West shoa	2540	13	208560	*	-	25	21163	East wollega	2340
2	20059	West shoa	1630	14	208571	*	-	26	21266	South wolo	2550
3	20068	West shoa	2010	15	208575	*	-	27	21278	South wolo	-
4	20163	East tigrai	2300	16	208584	*	-	28	213168	-	-
5	20168	East tigrai	-	17	208585	East shoa	1600	29	21369	Jimma	1720
6	208419	West gojam	2050	18	208594	East hararrgae	1750	30	214620	North omo	-
7	208507	*	-	19	208596	East hjarargae	-	31	215284	East gojam	-
8	208513	*	-	20	208865	North omo	1300	32	215562	Gedeo	1820
9	208523	*	-	21	208961	East wellega	2700	33	215790	West wolega	1950
10	208530	*	-	22	21057	East gojam	-	34	YD	Check	2400
11	208545	*	-	23	21068	Bale	2500	35	S-67	Check	2400
12	208558	*	-	24	21069	-	-	36	H-1	Check	2400

*Donation by foundation for agricultural plant breeding S.V.P P.O. Box 117 Wageningen, The Netherlands. -: Information not available.
Code: Genotype by code. Acc. No: Genotype accession number

6×6 simple lattice designs with two replications and with a plot size of 3×1.8 m. The spacing between sub-blocks was 2 m and plots within sub-blocks were 0.6 m. Each genotype was planted in a plot consisting of six rows of 3 m long with spacing of 30 cm between rows. The distance between replications was 2.5 m. All recommended agronomic practices (Alemayehu and Mesfin, 1994) were followed to raise good crop.

Data were collected on seed oil quality traits: Oil content was determined using Nuclear Magnetic Resonance Spectrometry (NMRS). It was measured as percentage of fat in the seed. A sampled 22 g of seeds was dried in an oven for 2½ h at 78°C and cooled for 30 min. Then, oil contents of seeds were measured using the procedure of Robbelen *et al.* (1989).

Fatty acid composition of the seed was determined using: Near Infrared Reflectance Spectroscopy (NIRS) using the procedure of Thies (1971). Major fatty acid compositions such as palmitic, stearic, oleic, linoleic, linolenic, eicosenoic and erucic acid were considered in the study. Each was measured as percentage of total fatty acid and was performed on 3 g of samples using Foss NIRS 5000 in the 1108-2492 ranges with an 8 nm step. The spectrum of each sample was taken by scanning (Win Scan) version 1.5, 2000, Intrasoftware international, L.L.C.).

Data analysis: Multivariate analyses such as cluster and principal component analyses of the genotypic values were computed using the procedures CLUSTER (ward's minimum) and PRINCOMP, respectively using SAS software version 9.00 (SAS, 2002). The genotypic values were determined as the method described by Zhu (1996) but considering the interaction component as nil (Falconer, 1981). Genetic distance between clusters was calculated using the generalized Mahalanobis' D^2 statistics using the equation:

$$D_{ij}^2 = (X_i - X_j)' S^{-1} (X_i - X_j)$$

where, D_{ij}^2 is the distance between groups i and j ; X_i and X_j are the vectors of the means of the traits for groups i and j and S^{-1} is the pooled within groups variance-covariance matrix.

RESULTS AND DISCUSSION

In any crop improvement venture, genetically distant parents are needed for crossing programs. This is to create the required genetic diversity between genotypes in terms of gene frequencies which may result for heterotic group and/or transgressive segregants. Univariate analysis of variance has shown that there was significant variation among genotypes in all traits which indicates existence of considerable genetic variability for selection and breeding (Table 2). Similarly, Alsemaan *et al.* (2011) reported the existence of genetic diversity within *Rosa damascena* cultivated in Syria and recommended Almarahl and Bab Alnayrab accessions to be used to broaden the production of rose oil. Further, Kalpesh and Mohan (2009) reported that the existence of genetic diversity among 25 medicinal climber of *Tinospora cordifolia* evaluated in India. On the other hand, Gichimu and Omondi (2010) reported that morphological characterization of five newly developed lines of arabica coffee as compared to commercial cultivars in Kenya. They reported low genetic variation among newly developed lines of arabica coffee as compared to commercial cultivars in Kenya and emphasizing the need to broaden the genetic base of Arabica coffee in Kenya.

Means of quality traits of the genotypes are presented in Table 3. The oleic, linoleic, linolenic, erucic and oil content of the genotypes ranged from 4.4-10.9, 15.3-19.6, 10-15.9, 38.6-51.8 and

39.8-47.7%, respectively. The highest value of oleic acid was shown by genotype 11 whereas the lowest was by genotype 21. Likewise, the highest value for erucic acid was recorded by genotype 7 and the lowest was recorded by genotype 11 which could be used as parental material for improvement of erucic acid content of the seed oil of Ethiopian mustard. Generally, these results indicate that those traits which had wide range of variations, will serve for breeding and selection for improvement of the trait desired. Erucic acid constitutes the major proportion of the total fatty acids which is in agreement with the findings of Teklewold (2005) and Genet *et al.* (2004). In this study the range of oil content of the genotypes is almost similar with the oil content of the released varieties (Alemayehu, 1990). In contrast of this study, a wide range of 25-48% oil content in Ethiopian mustard (*Brassica carinata*) germplasm was reported (Genet *et al.*, 2004).

Table 2: Mean squares of genotypes for eight quality traits

Traits	Error mean squares	Genotypes mean squares	Block mean squares	CV
Palmitic	0.053	0.227**	0.000001	6.78
Stearic	0.005	0.0214**	0.0227	6.52
Oleic	1.50	4.14**	0.0072	18.28
Linoleic	0.43	2.18**	2.0134	3.63
Linolenic	2.03	5.79**	3.38	11.38
Eicosenoic	4.18	8.71*	1.468	19.31
Erucic	3.17	15.95**	0.001485	3.85
OC	1.53	5.54**	0.05	2.78

*, **p = 0.05 and p = 0.01 significance level respectively, OC: Oil content

Table 3: Mean values of the studied 36 genotypes for 8 quality traits as determined by NIRS

Code	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Eicosenoic	Erucic	OC
1	3.85	1.15	5.37	18.94	9.99	13.21	48.33	42.85
2	3.53	1.22	4.90	16.67	10.42	8.09	48.57	42.45
3	3.86	1.15	6.07	17.74	10.80	12.80	49.14	43.40
4	3.26	1.02	6.67	18.82	13.36	10.17	45.67	45.35
5	3.53	1.13	7.12	17.93	10.46	11.88	47.77	43.40
6	3.60	0.76	4.60	19.47	14.37	7.34	47.89	44.55
7	4.44	1.03	5.79	17.96	13.96	13.21	51.81	41.00
8	3.77	1.16	5.43	18.41	10.05	12.65	49.22	43.35
9	3.29	0.93	6.54	18.12	12.38	10.48	46.78	46.20
10	3.26	1.13	9.14	16.59	12.05	15.55	41.86	43.30
11	3.36	1.05	10.89	19.33	15.12	8.91	38.61	42.00
12	3.37	1.20	7.08	16.20	10.24	12.61	46.27	43.40
13	3.01	0.88	6.85	17.51	15.87	7.50	42.99	39.75
14	3.88	1.07	5.52	16.74	10.08	14.20	50.48	42.35
15	3.55	0.91	6.09	18.19	12.31	10.18	48.82	45.00
16	3.56	1.01	6.55	18.25	11.31	10.61	48.00	43.20
17	2.82	1.16	9.41	15.71	10.47	12.53	42.44	45.00
18	3.36	0.97	6.14	18.23	12.34	9.38	47.67	43.80
19	3.12	1.20	7.61	15.33	10.70	9.47	44.39	45.05
20	2.91	1.12	8.94	17.96	11.27	9.25	41.03	44.80
21	3.62	1.03	4.37	18.72	15.60	8.14	48.54	43.85

Table 3: Continued

Code	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Eicosenoic	Erucic	OC
22	2.97	1.08	4.96	18.50	12.11	8.09	47.42	42.90
23	2.94	1.08	8.84	18.30	12.21	8.71	41.74	43.85
24	3.01	0.96	6.78	18.03	14.45	10.44	45.46	43.90
25	3.27	1.05	6.86	19.57	13.79	7.50	45.41	45.90
26	3.17	1.00	7.07	18.31	13.28	9.04	43.06	46.25
27	3.76	1.00	6.44	18.22	11.64	12.21	47.52	44.60
28	3.60	1.00	5.93	19.38	13.89	11.89	47.38	42.05
29	3.28	0.90	7.18	17.94	14.75	11.27	45.19	45.80
30	3.25	1.20	7.16	16.27	11.63	11.34	46.14	46.20
31	3.41	0.97	6.57	18.33	13.36	9.95	46.17	43.50
32	3.65	1.01	5.82	18.25	14.04	11.87	47.22	42.70
33	3.46	0.98	5.41	18.40	13.00	7.76	48.37	41.50
34	3.21	1.00	5.59	18.91	14.09	9.94	47.18	47.65
35	3.18	1.03	7.13	18.98	13.02	10.18	46.01	44.60
36	3.09	1.12	8.38	17.31	11.57	12.71	44.31	45.50
Range	2.8-4.4	0.8-1.2	4.4-10.9	15.3-19.6	10-15.90	7.3-15.6	38.6-51.8	39.8-47.7
Mean	3.39	1.05	6.70	17.99	12.50	10.59	46.25	43.92
SD	0.37	0.11	1.67	1.14	1.98	2.52	3.07	1.87

OC: Oil Content, SD: Standard deviation

Table 4: Pairwise generalized squared distance (D²) among 36 genotypes of Ethiopian mustard in seven clusters based on their quality traits

Cluster	C1	C2	C3	C4	C5	C6	C7
C1	0	43.2**	19.0*	26.9**	53.7**	35.3**	93.1**
C2		0	25.9**	10.4	149.4**	57.0**	15.2
C3			0	17.8*	93.9**	41.7**	51.3**
C4				0	122.4**	50.4**	34.2**
C5					0	84.8**	204.2**
C6						0	100.7**
C7							0

*, ** X² test *0.01 and 0.05 significance level respectively, C: Cluster

Clustering produced a clear grouping of the 36 genotypes into seven clusters, whereby the individuals within any one cluster are more closely related than are individuals in different clusters (Fig. 1). Similarly, individuals in clusters with non-significant genetic distance were presumed to have close relationships with each other than those in clusters with significant genetic distant. Rohman *et al.* (2004) reported that high divergence between the clusters for 25 sorghum varieties which were grouped into four clusters. The pairwise generalized squared distance (D²) among the clusters is presented in Table 4. Genetic distances among most clusters were significant. The highest genetic distance was recorded between C5 and C7 (204.2) followed by C2 and C5 (149.4) and C4 and C5 (122.4). The genetic divergence between C1 and C2, C1 and C4, C1 and C5, C1 and C6, C1 and C7, C2 and C3, C2 and C6, C3 and C5, C3 and C6, C3 and C7, C4 and C7, C5 and C6 were also highly significant from which parents may be selected for crossing in order to obtain genetic recombination and transgressive segregants in the subsequent generation. However, it is also valuable considering genotypes within a cluster with respect to a trait of interest as suggested by

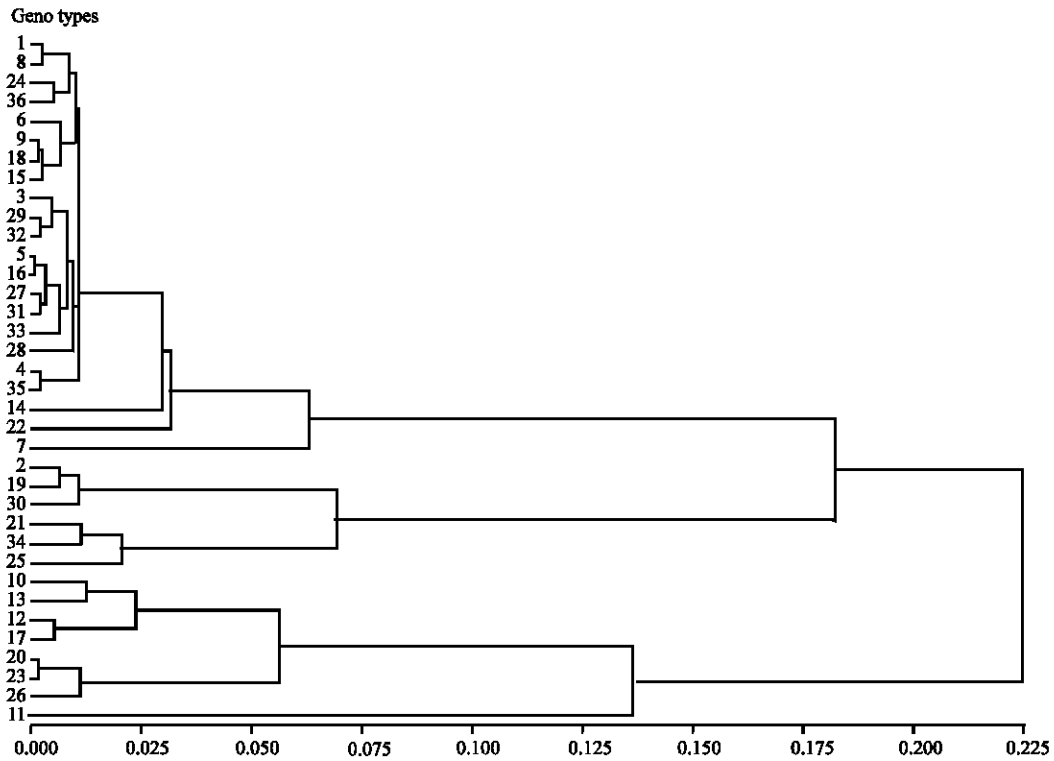


Fig. 1: Wards minimum variance dendrogram which shows the distribution of the 36 Ethiopian mustard genotypes

Teklewold *et al.* (2000), Chahal and Gosal (2002) and Keneni *et al.* (2005). Genetic distances between C2 and C4 and between C2 and C7 were non significant, indicating close relationships among the genotypes.

Pattern of distribution of genotypes in different clusters showed no definite correspondence between genetic diversity and geographic origin. For instance, genotypes 1 and 3 both from West Shewa, genotypes 4 and 5 both from East Tigray, genotypes 22 and 31 both from East Gojam were grouped in C1. This grouping of genotypes of the same origin in the same cluster may be as a result of their similar genetic background. On the other hand, there are also genotypes with same geographical origin but grouped in different clusters in which differential selection criterion, genetic drift and adaptation to different agro-climatic conditions might be the cause. Paradoxically, genotypes with different geographical origin were grouped in same cluster in which case synchronization of selection differential for different traits in different areas might have been occurred. The aforementioned phenomena have also been reported by Keneni *et al.* (2005), Verma and Sachan (2000), Jeena and Sheikh (2003) and Teklewold *et al.* (2000).

Solitary genotypes in C2 and C7 were among donations which have previously been collected from Ethiopia as *Brassica carinata*. This phenomenon might have resulted from geographical barriers preventing gene flow or intensive selection for adaptive gene complexes as have been suggested by Teklewold *et al.* (2000), Bhatt (1973) and Joshi and Singh (1979). Generally, donated materials were grouped in different clusters such as C1, C2, C5 and C7 which may be inferred that they may genetically affiliate from one of their respective cluster's member. Similarly, the two unknown genotypes such as genotypes 24 and 28 were grouped in the same cluster which

indicates that they may be closely related in their pedigree and/or the operation of similar forces of natural or artificial selection made them to be grouped together. S-67 and Holetta-1 grouped together in C1 but Yellow-Dodolla was identified in C4 which indicates that these varieties may have close pedigree relationships with the genotypes in their respective clusters. Similarly, Iftekharruddaula *et al.* (2002) reported that clustering pattern was not influenced by the geographic origin rather it was influenced by the pedigree of the breeding lines in rice. On the other hand, Zhuang *et al.* (2011) reported that the clustering analysis done on Persian Wheat (*Triticum turgidum* ssp. *carthlicum*) accessions using EST-SSR Markers suggested that most of the accessions with adjacent geographic origins had the tendency to cluster together.

The Intra-class average and range of genetic divergence of the seven clusters of Ethiopian mustard genotypes (Table 5) showed that C1 was the largest constituting 58.3% of the total genotypes in which wide range of values for the traits were found. High oleic and linoleic acid with low erucic acid content in their seed oil was shown by C7. On the other hand, C2 showed the highest erucic acid content in its seed oil. The highest oil content in their seed was revealed in C4.

In order to assess the patterns of variations, Principal Component Analysis (PCA) was done by considering eight quality traits simultaneously. Principal component analysis (Table 6) showed that

Table 5: Intra-class average and range of genetic divergence in quality traits of the seven clusters of Ethiopian mustard genotypes

Cluster	-----											
	C1		C2	C3		C4		C5		C6		C7
Traits	R	M	M	R	M	R	M	R	M	R	M	M
Palmitic	3-3.9	3.5	4.4	3.12-3.53	3.3	3.2-3.6	3.4	2.8-3.4	3.1	2.9-3.2	3.0	3.40
Stearic	0.8-1.2	1	1	1.2-1.22	1.2	1-1.1	1.0	0.9-1.2	1.1	1-1.1	1.1	1.10
Oleic	4.6-8.4	6.2	5.8	4.9-7.61	6.6	4.4-6.9	5.6	6.9-9.4	8.1	7.1-8.9	8.3	10.9
Linoleic	12.4-19.5	18	18	15.3-16.67	16.1	18.7-19.6	19.1	15.7-17.5	16.5	18-18.3	18.2	19.3
Linolenic	10-14.8	12.3	14	10.7-11.6	10.9	13.8-15.6	14.5	10.2-15.9	12.2	11.3-13.3	12.3	15.1
Eicosenoic	7.3-14.2	10.9	13.2	8.1-11.3	9.6	7.5-9.9	8.5	7.5-12.6	12.0	8.7-9.3	9.0	8.90
Erucic	44.3-50.5	47.4	51.8	44.4-48.6	46.4	45.4-48.5	47	41.9-46.3	43.4	41.0-43.1	41.9	38.6
OC	41.5-46.2	43.8	41	42.5-46.2	44.6	43.9-47.7	45.8	39.8-45	42.9	43.9-46.3	45	42.0

OC: Oil content, C: Cluster, R: Range, M: Mean

Table 6: Component scores of the first six principal components of 36 genotypes of Ethiopian mustard

Traits	Component score				
	1	2	3	4	5
Palmitic	0.389	0.388	-0.201	0.279	0.133
Stearic	-0.371	0.376	0.024	-0.252	0.494
Oleic	-0.495	-0.190	-0.335	0.320	0.124
Linoleic	0.389	-0.293	0.004	0.306	0.759
Linolenic	0.263	-0.470	-0.239	0.159	-0.302
Eicosenoic	-0.144	0.442	-0.171	0.682	-0.199
Erucic	0.438	0.384	0.250	-0.075	-0.128
OC	-0.181	-0.151	0.836	0.408	-0.025
Eigenvalue	2.70	2.50	1.00	0.80	0.50
Variance (%)	33.90	31.90	12.70	9.50	6.10
Cumulative (%)	33.90	65.70	78.40	87.90	94.00

OC: Oil content

94.0% of the variation was contributed by the first five principal components. 33.9% of the variation was depicted by the first principal component in which erucic, palmitic, linoleic and linolenic acid were the major positive contributors. In this principal component, stearic and oleic had negative weight. Additional variation of 31.8% was revealed by the second principal component which accounted positively mainly for stearic acid. Linolenic acid had the highest negative weight in this principal component. Another additional variation of 12.7, 9.5 and 6.1% were shown by the third, fourth and fifth principal component, respectively.

Oil content in the third, eicosenoic and oleic in the fourth and linoleic and stearic in the fifth principal component were the major positive contributors. In general, it is assumed that traits with larger absolute values closer to unity within the first principal component influence the clustering more than those with lower absolute values closer to zero (Chahal and Gosal, 2002). Accordingly, most of the traits individually contributed small effects ($\pm 0.18-0.49$) to the total variation and principally these aggregate effects which were responsible for cluster formation. Nevertheless, those traits which had relatively greater weight in the first principal component had higher relative contribution to the total diversity and responsible for cluster formation.

CONCLUSIONS

Multivariate analysis of genetic divergence among genotypes has resulted in the formation of seven clusters and showed the existence of adequate genetic variation for further selection and breeding. Significant genetic distances between most clusters were observed from which selection of parents may be made for crossing in order to obtain genetic recombination and transgressive segregants. Parental material for improvement of seed oil of Ethiopian mustard may be obtained from those genotypes which have high oleic but low erucic acid content in the seed oil in cluster 7. Genotype in cluster 2 has shown highest erucic acid content in its seed oil which may be used as sources of genes in efforts of breeding for industrial purposes. Genotypes constituted in cluster 4 showed high oil content in their seed which could also be exploited during breeding for seed oil content. Clustering of genotypes into groups was mainly attributed by cumulative effects of individual traits. In general, this study indicates that there is a possibility of improving the fatty acid profile as well as the oil content of the genotypes through further breeding endeavor such as inter population crossing. The present investigation also revealed that diverse geographic origins of the genotypes could not necessarily be an index of variation and the factors other than geographic diversity such as genetic drift, selection pressure and environment may be responsible for discrepancy of genotypes.

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