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Full Length Research Paper

Genetic relationships among Ethiopian mustard genotypes based on oil content and fatty acid composition

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Quantification and classification of genetic diversity among genotypes is essential for parental selection in breeding programs. The objective of this study was to classify and cluster Ethiopian mustard genotypes according to their fatty acid composition, and to assess the genetic relationship between the genotypes. This study revealed wide variation in fatty acid composition. Principal component analysis showed that desaturation ratio, elongation ratio, monounsaturated fatty acids, oleic desaturation ratio, and vaccinic acid had the highest loading in the first component that accounted for 39.28% of the total variation. For the second principal component stearic acid, saturated fatty acid, palmitic acid, oleic desaturation ratio, polyunsaturated fatty acids, and α-linolenic acid had the highest loading that accounted for 30.97% of the total variation. Five principal components explained 96.01% of the total variation. The dendrogram generated by the UPGMA cluster analysis grouped *B. carinata* genotypes into 11 distinct clusters. The pair-wise mean genetic distance estimates based on fatty acid composition was 1.08 ± 0.02. The information generated from this study can be used to plan crosses and maximize the use of genetic diversity and expression of heterosis.

Key words: *Brassica carinata*, capillary gas chromatography, fatty acids, genetic diversity, oil content.

INTRODUCTION

Plant seeds are sources of oils of nutritional, industrial and pharmaceutical importance. The suitability of oil for a particular purpose, however, is determined by its fatty acid composition. No oil from any single source has been found to be suitable for all purposes because oils from different sources generally differ in their fatty acid composition (Dagne and Jonsson, 1997). This necessitates a continuous search for new genetic resources of useful novel oils. As a result, to date the seed oil of several thousand plant species has been chemically analyzed and a few of these plant species are taken into cultivation as a new oil crop (Hirsinger, 1989). The patterns of fatty acid variation among groups of plant species also proved to be useful in chemotaxonomy and phylogenetic studies (Vickery, 1971; Rogers, 1972; Opute, 1978; Graham et al., 1981; Plessers, 1966; Yermanos et al., 1966; Velasco and Goffman, 2000).

Another approach to introduce novel oils is to develop new cultivars from established oil crops for innovative end-uses. The low and high erucic acid oil producing *Brassica* cultivars for human consumption and industrial

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Table 1. List of Ethiopian mustard (*B. carinata* A. Braun) accessions used in this study.

Code	Accession	Code	Accession	Code	Accession
1	Yellow Dodolla-03	34	Adet	67	PGRC/E 21361-03
2	S-67-02	35	PGRC/E 21358/2	68	PGRC/E 21261-04
3	PGRC/E 21261-03	36	PGRC/E 207928	69	PGRC/E 21001
4	PGRC/E 203221-03	37	PGRC/E 21320/5	70	PGRC/E 21261-01
5	C94-S-67	38	PGRC/E 20112/2	71	PGRC/E 21324
6	C94-Dodolla	39	PGRC/E 20165	72	PGRC/E 21163/1
7	Yellow Dodolla-01	40	PGRC/E 21051	73	PGRC/E 20163
8	PGRC/E 203221-01	41	PGRC/E 21261-05	74	PGRC/E 21324/1
9	PGRC/E 21261-02	42	PGRC/E 210406	75	PGRC/E 21236/1
10	PGRC/E 20130	43	PGRC/E 21169	76	PGRC/E 20104
11	Merawi	44	PGRC/E 21184	77	PGRC/E 21170
12	PGRC/E 20059	45	PGRC/E 20113	78	PGRC/E 208410
13	PGRC/E 21207	46	PGRC/E 20021	79	PGRC/E 207931
14	PGRC/E 20080	47	PGRC/E 21252	80	PGRC/E 208004
15	PGRC/E 207929	48	PGRC/E 20126	81	PGRC/E 21304
16	PGRC/E 207975	49	PGRC/E 21163	82	PGRC/E 21010
17	PGRC/E 20168/1	50	PGRC/E 20013	83	PGRC/E 21031
18	PGRC/E 20095/1	51	PGRC/E 21057/1	84	PGRC/E 20153
19	PGRC/E 21356/1	52	PGRC/E 20076/3	85	PGRC/E 20126/1
20	PGRC/E 21237	53	PGRC/E 20168/2	86	PGRC/E 20120
21	PGRC/E 21156	54	PGRC/E 200394	87	PGRC/E 208551
22	PGRC/E 20112/2	55	PGRC/E 207481	88	PGRC/E 20103
23	PGRC/E 200413	56	PGRC/E 21172	89	PGRC/E 21373
24	(4DxZem-1) X (Zem-1-AD/88)	57	PGRC/E 20165/1	90	PGRC/E 20164
25	(4DxZem-1) X (Zem-1-F5/10)	58	PGRC/E 208401	91	PGRC/E 20165/2
26	PGRC/E 20156	59	PGRC/E 21223/2	92	PGRC/E 20090/1
27	PGRC/E 21162/1	60	PGRC/E 21224/3	93	PGRC/E 20162/1
28	PGRC/E 20163/1	61	PGRC/E 20168/3	94	PGRC/E 21169/1
29	PGRC/E 20076/2	62	PGRC/E 21328/1	95	PGRC/E 20175/1
30	PGRC/E 20147/1	63	PGRC/E 21162	96	PGRC/E 21058/2
31	PGRC/E 208404	64	PGRC/E 21235/1	97	PGRC/E 21261
32	Yellow Dodolla	65	PGRC/E 21263	98	PGRC/E 20163/5
33	S-67-01	66	PGRC/E 21057		

purposes, respectively, is one such example (Downey and Röbbelen, 1989).

Ethiopian mustard (*Brassica carinata* A. Braun; n=2x=17 BBCC) is a high yielding oilseed crop of the Ethiopian highlands. In its native Ethiopia, it is used as leaf vegetable and as an oilseed. The seed oil of *B. carinata* is high in erucic acid (≈ 40% of the total fatty acids) and is therefore undesirable for human consumption (Getinet et al., 1994). Oils containing high amounts of erucic acid are suitable for industrial applications. Therefore, both developments of commercial varieties free of erucic acid content and with very high erucic acid content are breeding objectives in *Brassica* oil crops. Other important objectives are the increase of oleic acid, the increase of linoleic acid, and the reduction of linolenic acid content (Röbbelen, 1991).

For this reason, the characterization of germplasm collections for seed oil composition now has special importance, with a view to identify potentially improved genotypes. For the choice of diverse parents for a hybridization program, multivariate analysis using principal component and cluster analysis has been extensively used as a quantitative measure of genotypic divergence among the parents. Therefore, an attempt has been made to study genetic diversity among 98 genotypes of Ethiopian mustard based on oil content and fatty acid composition.

MATERIALS AND METHODS

Plant material

A total of 98 accessions of *B. carinata*, obtained from the Ethiopian National Breeding Program and from the germplasm collections of the Institute of Biodiversity Conservation and Research, Addis Ababa, Ethiopia and two accessions from Canada were used in this study. The germplasm collections represented the major *B. carinata* growing regions of the country. A detailed description of the materials used in this study is shown in Table 1. The plants were grown in the fields of Adet Research Center in the 1999/2000 main cropping seasons. Each sample consisted of seeds from different plants of each accession, which were analyzed for seed oil content and fatty acid composition.

Lipid extraction

Total lipid was extracted with chloroform-methanol (2:1 v/v) as described by Folch et al. (1957). Butylated hydroxy toluene (20 g) was placed in a 2000 ml volumetric flask. Chloroform (1333.33 ml) and methanol (666.67 ml) were added. In the lower phase, chloroform (1700.97 ml) was placed in 2000 ml volumetric flask, methanol (277.23 ml) and water (19.8 ml) were added (86:14:1, v/v/v), respectively. About 200 mg seeds from each sample were ground with mortar and pestle. For lipid extraction 0.5 g ground seed was used in a 250 ml round bottom flask 19, 30 ml of chloroform : methanol (2:1, v/v) was added and left overnight in a refrigerator at 4°C.

The sample was transferred into a round bottom flask through a pre-weighed 18.5 cm Whatman No. 1 filter paper on top of the separating funnel. The round bottom flask was washed three times with 12.33 ml of chloroform : methanol (2:1, v/v) and transferred each time to filter paper. After allowing everything to run through, the filter paper was removed. Then 16.5 ml distilled H_2O was added to each separating funnel, which was recapped, and shaken thoroughly, and allowed 1 h for separation. After this, the lower phase was drained into a 500 ml round bottom flask and 50 ml of lower phase was added to each separating funnel and allowed 15 min for separation. After the 15 min, the lower phase was drained into the same 500 ml round bottom flask and the upper phase discarded. Contents from the 500 ml of round bottom flask were evaporated under vacuum in a rotary evaporator at 60ºC for 20 min at 15 psi. The remaining water was removed by the addition of 30 ml methanol and then by evaporating each round bottom flask at 60ºC at 15 psi. Contents of each 500 ml round bottom flask were washed six times with 5 ml portions of diethyl ether into a pre-weighed polytop. Diethyl ether was removed from each polytop by evaporation under a stream of nitrogen on a heating block at 60ºC for 20 min. Filter papers and capped polytops were put in a vacuum oven, dried at 50ºC overnight and weighed the next morning. The oil-solvent mixture was dried over anhydrous sodium sulphate, the solvent evaporated under nitrogen, and the oil percentage determined by weighing. 10 mg of the lipid was transferred to another polytop for methylation.

Methylation

A modification of the procedure described by Slover and Lanza (1979) was used. An amount of 10.0 mg of lipid was washed with 6 x 1 ml hexane into the test tube with a Teflon-lined cap. Hexane was removed by N2 evaporation without application of heat. Methanolic 0.5 N NaOH (1 ml) was added, and the tube capped and heated in a boiling water bath for 15 min. After the tube had cooled, 2 ml of BF3/CH3OH (14%) was added; then the tube was recapped and heated in the boiling water bath for an additional 15 min. The tube was cooled, and 1 ml of hexane and 2 ml of saturated aqueous NaCl were added. The tube was shaken vigorously for 1 min, and allowed to stand for 10 min until the phases separated. The upper 70% of hexane layer was transferred with a Pasteur pipette to a 45 x 11 mm vial containing a 1 mm layer of anhydrous Na2SO4. The vial was capped, shaken, and allowed to stand for at least 20 min to remove traces of water, then 100 µl hexane from each vial was transferred to a clean labeled auto-sampler vial and 900 µl hexane was added to each vial and stored below freezing point for GC analyses.

Determination of fatty acid composition

After methylation, individual fatty acid composition was determined by gas chromatography for all accessions. Fatty acids were quantified using a Varian GX 3400 flame ionization gas chromatograph, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 µm ID, 0.2 µm film thickness). Column temperature was 40-230ºC (hold 2 min 4ºC/min; hold 10 min). Fatty acid methyl esters in hexane (1 µl) were injected into the column using a Varian 8200 CX Auto-sampler with a split ratio of 100:1. The injection port and detector were both maintained at 250ºC. Hydrogen was used as a carrier gas at 45 psi and nitrogen was used as the makeup gas. Chromatograms were recorded with Varian Star Chromatography Software version 4.0. Identification of sample fatty acids was made by comparing the relative retention times of fatty acid methyl ester peaks from samples with those of standards obtained from SIGMA (cat. 189-19).

Statistical analysis

The statistical package NCSS (Jerry 2001) was used for the following statistical procedures:

Principal Component Analysis: Principal component analysis (PCA) is a data analysis tool that is usually used to reduce the dimensionality (number of variables) of a large number of interrelated variables, while retaining as much of the information (variation) as possible. PCA calculates an uncorrelated set of variables (factors or pc's). These factors are ordered so that the first few retain most of the variation present in all of the original variables. The computation of PCA reduces to an eigenvalue-eigenvector problem. PCA is a data analytical, rather than statistical, procedure.

NCSS statistical software (Jerry, 2001) was used to perform PCA with the adjusted data matrix, x, which consists of n observations (rows) on p variables (columns). The adjustment is made by subtracting the variable means from each value. That is, the mean of each variable is subtracted from all of that variable value. This adjustment is made, since PCA deals with covariances among the original variables, so the means are irrelevant.

New variables are constructed as weighted averages of the original variables. The new variables are called the factors, latent variables, or principal components. Their specific values on a specific row are referred to as the factor scores, the component scores, or simply the scores (Jerry, 2001).

Genetic distance calculations: Distance matrices for all pairs of genotypes were constructed from the interval and ratio lipid data using the Euclidean distance method (Jerry, 2001). The Euclidean distances are the square roots of the sum of squares of the distances between the multidimensional space values of the variables for any two genotypes.

Cluster analysis: Cluster analysis was performed based on the genetic distance matrices generated by the Euclidean distance method to reveal the patterns of genetic relationships among genotypes. Several clustering algorithms were tried. However, the unweighted pair group method using arithmetic averages (UPGMA) (Jerry 2001) minimizes within-cluster variance, and appeared to give the most satisfactory clustering result. The results of cluster analysis were presented in the form of dendrograms to depict degree of similarity and to infer relationships among genotypes. The cophenetic correlation (Jerry 2001) for each dendrogram was also computed as a measure of 'goodness of fit' for the method of cluster analysis used. Cluster analysis can be used to identify accessions with similar adaptation, which can be useful for

Table 2. Fatty acid profiles of Ethiopian mustard (*B. carinata*) accessions/varieties determined by capillary gas chromatography.

No	Accessions/ varieties	%Fat	Palmitic C16:0	Stearic C18:0	Vaccinic C18:1c7	Oleic C18:1c9	Linoleic C18:2c9,12	ALA C18:3c9 12,15	Eicosenoic C20:1c11	Erucic C22:1c 13	ER	DR	ODR	LDR	EFA	SFA	MUFA	PUFA
1	Yellow Doddolla-03	41.85	4.11	1.41	1.21	9.44	23.38	17.33	7.68	29.25	41.83	46.11	79.27	42.57	40.72	6.84	48.76	43.64
2	S-67-02	40.14	4.17	1.55	1.48	9.78	24.58	16.33	7.10	27.99	40.21	46.88	78.41	39.92	40.91	7.07	47.59	43.98
3	PGRC/E 21261-03	37.36	4.09	1.68	1.39	9.49	21.76	17.75	6.30	30.84	42.43	45.14	78.41	44.92	39.51	7.12	49.20	42.62
4	PGRC/E 203221-03	41.15	5.79	1.90	2.66	16.36	26.55	19.36	8.74	13.32	25.34	52.81	70.74	42.26	45.91	8.77	42.01	47.79
5	C94-S-67	24.92	4.64	1.96	2.85	19.74	28.51	22.20	5.91	8.73	16.63	57.71	69.22	43.9	50.71	7.76	38.09	52.19
6	C94-Dodolla	34.98	5.48	1.77	3.10	21.59	28.53	22.52	4.90	6.91	13.48	58.36	67.45	44.21	51.05	8.32	37.32	52.27
7	Yellow Dodolla-01	37.52	3.93	1.52	1.40	9.93	20.31	17.80	8.49	30.82	44.3	42.94	77.08	46.71	38.11	6.71	51.99	41.03
8	PGRC/E 203221-01	42.04	3.94	1.47	1.71	12.30	22.59	19.12	8.20	25.33	37.57	46.73	74.85	45.84	41.71	6.48	48.55	44.35
9	PGRC/E 21261-02	47.97	4.22	1.54	1.51	11.42	21.47	19.45	7.67	27.06	39.2	46.19	75.98	47.54	40.92	6.86	48.82	43.39
10	PGRC/E 20130	40.94	4.05	1.51	1.59	10.29	22.38	18.13	7.87	27.88	40.56	45.97	77.34	44.76	40.51	6.72	48.92	43.61
11	Merawi	41.92	4.29	1.61	1.66	12.11	21.43	20.10	7.77	24.70	36.95	47.37	75.13	48.49	41.53	7.09	47.45	44.14
12	PGRC/E20059	41.88	3.54	1.22	1.58	9.53	19.10	19.72	8.12	30.55	43.64	43.82	77.76	50.8	38.83	5.96	51.02	42.09
13	PGRC/E 21207	39.36	4.19	1.43	1.53	9.72	21.84	19.04	7.28	28.36	40.61	46.58	78.44	46.58	40.89	7.03	48.12	44.14
14	PGRC/E 20080	39.41	4.14	1.57	1.58	10.22	21.72	18.75	7.60	28.03	40.54	46.04	77.42	46.33	40.47	6.86	48.68	43.29
15	PGRC/E 207929	40.12	4.22	1.64	0.96	14.55	19.84	19.32	8.60	25.17	38.15	44.33	71.68	49.43	39.17	7.20	50.28	41.17
16	PGRC/E 207975	40.50	3.68	1.71	1.32	12.72	20.29	19.22	8.76	27.25	40.21	44.11	73.78	48.64	39.50	6.64	50.94	41.87
17	PGRC/E 20168/1	35.18	4.32	1.49	1.89	10.65	22.12	17.97	8.29	27.26	40.32	45.46	76.18	44.82	40.09	6.97	49.36	43.01
18	PGRC/E 20095/1	38.43	4.01	1.50	1.44	9.25	20.58	17.80	8.01	29.90	43.55	44.17	78.25	46.47	38.38	6.97	49.94	41.56
19	PGRC/E 21356/1	42.78	4.10	1.53	1.50	12.77	20.95	18.50	8.05	25.92	38.71	45.03	73.47	46.99	39.45	6.98	49.28	41.64
20	PGRC/E 21237	39.48	4.77	1.63	1.61	10.05	22.18	19.36	7.50	26.66	39.07	47.6	78.12	46.71	41.55	7.66	47.07	44.32
21	PGRC/E 21156	38.03	3.87	1.50	1.47	10.37	22.25	17.80	7.95	28.68	41.38	45.24	77.18	44.44	40.04	6.69	49.69	42.98
22	PGRC/E 20112/2	40.09	3.74	1.36	1.52	10.06	21.71	17.89	7.97	29.49	42.22	44.72	77.41	45.28	39.60	6.25	50.31	42.697
23	PGRC/E 200413	41.87	3.68	1.71	0.86	12.80	20.58	18.49	8.72	26.79	40.2	44.33	74.14	47.44	39.07	6.80	50.14	41.52
24	(4DXZem-1) X (Zem-1-AD/88)	39.42	4.28	1.52	1.61	11.12	22.83	18.22	7.48	26.95	39.03	46.53	76.32	44.39	41.05	7.01	48.36	43.79
25	(4DXZem-1) X (Zem-1-F5/10)	39.93	4.53	1.62	1.68	11.74	23.58	18.70	7.60	24.80	36.77	47.99	75.9	44.24	42.28	7.34	46.86	44.93
26	PGRC/E 20156	37.89	4.28	1.53	1.63	11.71	22.77	19.11	7.89	25.52	37.69	47.25	75.83	45.63	41.87	6.98	47.83	44.34
27	PGRC/E 21162/1	39.21	3.98	1.40	1.69	10.15	21.52	17.68	7.92	29.32	42.19	44.4	76.8	45.11	39.19	6.50	50.47	42.19
28	PGRC/E 20163/1	42.23	3.94	1.57	1.42	11.50	20.73	19.99	8.38	26.64	39.51	45.92	75.91	49.09	40.72	6.77	49.03	43.33
29	PGRC/E 20076/2	38.09	3.92	1.37	1.37	9.88	21.76	18.86	7.52	29.03	41.33	45.94	78.32	46.43	40.62	6.56	48.97	43.56
30	PGRC/E 20147/1	30.87	4.13	1.36	1.46	11.07	22.20	18.44	7.40	28.22	40.11	45.77	76.43	45.37	40.64	6.61	49.35	43.33
31	PGRC/E 208404	42.00	4.26	1.77	1.60	12.88	23.35	17.89	8.92	23.45	36.75	46.82	74.02	43.37	41.24	7.27	47.68	43.49

Table 2. contd.

32	Yellow Dodolla	43.23	4.18	1.63	1.47	10.95	22.85	18.27	8.15	27.16	39.74	46.28	76.8	44.43	41.11	6.90	48.75	43.83
33	S-67-01	38.67	4.01	1.38	1.61	10.90	23.24	18.60	7.37	27.30	38.95	47	76.98	44.47	41.84	6.44	48.44	44.74
34	Adet	40.38	4.25	1.76	1.72	11.32	22.34	17.67	8.49	26.05	39.43	45.68	75.42	44.16	40.01	7.25	48.71	42.57
35	PGRC/E 21358/2	43.52	4.05	1.49	1.44	13.16	20.36	18.03	8.76	27.68	40.74	42.93	72.44	46.97	38.39	6.76	52.04	40.59
36	PGRC/E 207928	41.38	3.74	1.65	1.38	13.31	18.13	18.64	8.98	28.24	41.94	41.5	71.48	50.78	36.77	6.67	52.98	39.05
37	PGRC/E 21320/5	38.98	3.95	1.56	1.46	12.32	21.32	18.43	8.05	26.84	39.44	44.99	74.28	46.46	39.75	6.77	49.82	42.31
38	PGRC/E 20112/2	40.98	4.29	1.57	1.64	10.23	21.27	19.96	7.71	27.65	39.97	46.62	77.65	48.41	41.24	6.96	48.36	44.16
39	PGRC/E 20165	43.88	4.68	1.77	1.59	9.80	21.83	19.29	6.94	25.49	38.15	48.46	78.35	47.01	41.12	7.86	45.07	43.99
40	PGRC/E 21051	39.23	4.44	1.57	1.61	12.13	23.90	17.98	8.69	23.00	36.27	48.01	75.33	43.04	41.88	7.35	46.51	44.35
41	PGRC/E 21261-05	40.00	4.09	1.54	1.62	11.33	24.39	17.30	8.51	26.24	38.88	46.64	76.31	41.5	41.69	6.82	48.58	44.43
42	PGRC/E 210406	40.46	4.17	1.48	1.49	10.97	21.84	19.18	8.33	26.93	39.73	46.22	76.7	46.76	41.02	6.82	48.75	43.65
43	PGRC/E 21169	41.14	5.07	1.77	1.83	13.59	23.97	17.86	8.01	22.44	34.69	47.74	73.09	42.8	41.83	8.00	46.96	44.11
44	PGRC/E 21184	41.58	4.37	1.62	1.79	11.10	24.07	18.29	7.87	24.82	37.18	48.17	76.67	43.18	42.36	7.23	46.71	45.22
45	PGRC/E 20113	35.58	3.63	1.41	1.55	10.04	20.72	17.90	8.25	30.03	43.26	43.65	76.92	46.34	38.62	6.22	51.33	41.64
46	PGRC/E 20021	38.78	4.71	1.60	1.79	11.75	23.98	18.86	7.61	23.10	35.24	49.23	76.02	44.12	42.84	7.55	45.40	45.41
47	PGRC/E 21252	38.12	6.21	1.44	1.94	11.65	25.34	19.66	6.72	20.00	31.29	52.79	76.83	43.78	44.99	8.74	41.49	47.38
48	PGRC/E 20126	38.22	3.81	1.27	1.40	10.40	21.96	18.25	8.44	28.56	41.53	45.23	77.35	45.52	40.20	6.16	50.11	43.19
49	PGRC/E 21163	38.55	3.97	1.43	1.53	11.00	23.13	17.44	8.04	27.14	39.85	45.97	76.41	42.99	40.58	6.51	48.93	43.38
50	PGRC/E 20013	37.70	5.31	2.15	1.66	11.70	22.00	18.67	6.29	23.44	35.46	48.61	75.31	46.01	40.67	10.44	44.23	43.03
51	PGRC/E 21057/1	41.78	4.08	1.57	1.61	12.94	19.48	18.95	8.48	27.39	40.37	43.25	72.53	49.32	38.43	6.79	51.54	40.81
52	PGRC/E 20076/3	43.87	4.12	1.53	1.33	8.91	20.94	19.55	7.44	29.26	41.98	46.32	79.82	48.28	40.48	6.93	48.11	43.67
53	PGRC/E 20168/2	38.45	3.99	1.53	1.75	10.41	22.43	17.55	7.88	27.46	40.34	45.78	76.73	44.07	39.98	6.76	48.87	43.11
54	PGRC/E 200394	37.16	3.79	1.52	1.50	11.70	21.17	17.48	8.70	28.20	41.55	43.6	74.58	45.32	38.65	6.55	51.23	41.35
55	PGRC/E 207481	39.90	3.63	1.64	1.28	10.48	20.56	16.87	1.22	30.51	39.21	46.26	76.1	45.07	37.43	6.62	44.69	40.29
56	PGRC/E 21172	42.64	3.75	1.51	1.43	12.05	21.54	17.78	8.93	28.16	41.26	43.74	74.47	45.22	39.31	6.54	51.51	41.84
57	PGRC/E 20165/1	41.55	4.16	1.60	1.41	10.47	20.18	18.31	7.85	29.59	42.64	43.83	76.41	47.58	38.49	7.14	50.48	41.39
58	PGRC/E 208401	33.14	3.53	1.63	1.33	12.23	19.43	16.68	10.24	29.38	44.36	40.44	72.69	46.19	36.11	6.59	54.16	38.52
59	PGRC/E 21223/2	40.18	4.75	1.44	1.63	11.94	23.08	18.54	7.39	24.38	36.50	47.9	75.44	44.64	41.62	7.50	46.50	44.03
60	PGRC/E 21224/3	36.17	3.59	1.37	1.48	9.01	20.66	16.77	7.97	31.92	45.43	42.62	78.11	44.81	37.43	6.27	51.89	40.75
61	PGRC/E 20168/3	39.00	4.57	1.55	1.27	10.97	21.91	19.04	7.49	27.32	39.53	46.58	77.02	46.59	40.95	7.38	48.12	43.61
62	PGRC/E 21328/1	39.09	4.16	1.73	1.79	11.75	23.01	17.89	9.26	24.73	38.44	46.25	75.13	43.74	40.89	7.19	48.54	43.58
63	PGRC/E 21162	26.78	4.19	1.50	1.55	12.22	21.11	17.96	8.58	27.09	40.30	44.14	73.94	45.97	39.06	6.94	50.57	41.65
64	PGRC/E 21235/1	43.47	3.81	1.50	1.41	10.39	19.82	18.30	8.58	30.11	43.66	43.02	76.36	48.01	38.12	6.72	51.67	40.84
65	PGRC/E 21263	38.38	4.24	1.53	1.65	9.77	22.08	18.66	7.79	28.09	40.75	46.27	78.11	45.79	40.74	7.07	48.53	43.74

Table 2. contd.

66	PGRC/E 21057	38.88	3.96	1.39	1.73	9.20	21.68	18.28	7.43	29.56	42.09	45.47	78.52	45.74	39.96	6.57	49.40	43.18
67	PGRC/ E 21361-03	34.98	4.80	1.64	1.59	9.41	24.91	17.60	6.84	26.40	38.32	49.01	79.45	41.40	42.51	7.76	45.42	45.57
68	PGRC/ E 21261-04	36.50	4.61	1.82	1.99	10.38	22.63	20.65	6.70	25.37	36.56	49.34	77.77	47.71	43.29	7.69	45.41	46.13
69	PGRC/E 21001	39.45	3.95	1.41	1.68	10.94	23.01	19.32	7.52	26.75	38.42	47.44	77.04	45.64	42.33	6.34	48.02	45.15
70	PGRC/ E 21261-01	43.32	4.01	1.52	1.45	9.67	23.01	17.88	7.58	28.76	41.14	46.28	78.62	43.72	40.88	6.83	48.55	43.94
71	PGRC/E 21324	40.64	4.16	1.63	1.76	12.81	23.25	20.36	8.82	22.29	34.84	48.84	74.96	46.69	43.61	6.84	46.64	46.21
72	PGRC/E 21163/1	41.80	3.96	1.33	1.56	10.94	22.35	18.19	8.04	27.86	40.36	45.58	76.43	44.87	40.54	6.29	49.63	43.29
73	PGRC/E 20163	39.20	4.79	1.43	1.65	9.55	22.84	19.89	7.06	25.36	37.51	49.53	79.26	46.63	42.73	7.51	44.82	45.66
74	PGRC/E 21324/1	42.10	4.17	1.62	1.64	13.60	22.41	18.73	9.99	22.51	36.56	46.29	72.96	45.52	41.15	6.97	48.71	43.56
75	PGRC/E 21236/1	44.67	3.91	1.49	1.35	11.47	20.25	20.34	8.18	27.98	40.36	45.33	76.01	50.11	40.59	6.46	49.93	43.07
76	PGRC/E 20104	35.07	16.42	7.43	1.28	24.64	17.21	10.50	4.36	11.59	22.91	39.86	51.7	37.98	27.70	25.55	43.47	29.19
77	PGRC/E 21170	39.82	4.18	1.38	1.57	10.97	22.38	19.05	7.83	27.27	39.41	46.52	76.77	45.98	41.43	6.62	48.78	44.26
78	PGRC/E 208410	41.88	3.68	1.49	1.41	11.54	20.71	17.72	9.34	28.73	42.56	42.96	74.79	46.11	38.43	6.36	51.98	41.09
79	PGRC/E 207931	41.32	4.31	1.44	1.44	12.29	21.35	18.50	7.72	26.02	38.61	45.69	74.42	46.53	39.85	7.11	48.55	42.14
80	PGRC/E 208004	41.30	4.25	1.57	1.69	13.91	24.38	16.01	10.15	22.59	36.89	45.53	72.14	39.64	40.39	6.94	49.27	42.73
81	PGRC/E 21304	42.54	4.21	1.46	1.43	11.43	21.96	19.30	8.17	26.38	38.96	46.53	76.24	46.78	41.26	6.83	48.44	43.93
82	PGRC/E 21010	43.50	3.38	1.32	1.30	10.31	17.38	15.31	9.13	35.01	49.91	36.96	73.8	46.83	32.69	6.06	57.05	35.72
83	PGRC/E 21031	41.21	4.04	1.86	1.73	10.20	21.84	17.32	7.78	27.61	40.91	45.28	76.64	44.24	39.16	7.34	48.66	42.05
84	PGRC/E 20153	37.36	4.31	1.50	1.49	10.53	22.51	17.48	7.98	27.89	40.80	45.54	76.92	43.79	39.98	7.07	49.10	42.81
85	PGRC/E 20126/1	36.02	4.58	1.41	1.73	11.43	24.08	18.74	7.48	23.42	35.53	49.33	76.52	43.85	42.82	7.25	45.15	45.38
86	PGRC/E 20120	34.84	4.08	1.44	1.56	9.42	21.76	17.72	7.92	29.29	42.41	45.07	78.26	44.99	39.47	6.81	49.53	42.72
87	PGRC/E 208551	35.33	4.05	1.46	1.59	9.96	21.68	17.35	7.52	29.83	42.48	44.39	77.16	44.46	39.03	6.82	50.31	41.99
88	PGRC/E 20103	37.08	4.64	1.73	1.68	11.91	22.85	17.78	8.44	24.21	37.56	46.81	74.97	43.84	40.63	7.64	47.42	43.20
89	PGRC/E 21373	37.04	4.01	1.42	1.66	11.28	23.16	18.53	8.41	26.38	38.91	46.62	76.32	44.44	41.69	6.63	48.69	44.49
90	PGRC/E 20164	35.09	4.74	1.45	1.74	10.23	23.97	18.60	7.01	24.75	36.77	49.38	78.09	43.79	42.57	7.55	45.05	45.32
91	PGRC/E 20165/2	38.36	4.29	1.50	1.61	10.50	22.28	18.49	8.11	27.38	40.16	46.14	77.10	45.34	40.77	6.91	48.78	43.73
92	PGRC/E 20090/1	39.27	4.13	1.58	1.56	9.59	22.66	17.79	7.80	28.41	41.24	46.07	78.39	43.98	40.45	6.90	48.69	43.44
93	PGRC/E 20162/1	38.80	4.19	1.70	1.73	9.48	22.46	17.96	7.11	27.73	40.29	46.75	78.29	44.43	40.42	7.27	47.45	43.58
94	PGRC/E 21169/1	41.92	3.54	1.47	1.36	12.39	18.76	18.69	9.18	29.82	43.23	41.51	73.13	49.90	37.45	6.24	53.67	39.82
95	PGRC/E 20175/1	37.69	4.57	1.59	1.85	10.43	24.22	18.80	7.42	25.31	37.17	48.88	77.8	43.70	43.03	7.15	46.14	45.91

Table 2. contd.

96	PGRC/E 21058/2	40.52	3.85	1.52	1.30	9.53	19.80	17.04	8.25	31.86	45.70	41.97	77.29	46.26	36.84	6.85	52.25	39.85
97	PGRC/E 21261	40.36	4.22	1.53	1.48	11.23	23.39	17.15	8.13	27.60	40.16	45.56	76.14	42.31	40.54	6.88	49.47	43.40
98	PGRC/E 20163/5	42.10	3.78	1.56	1.30	10.35	19.85	19.00	8.50	29.62	43.02	43.84	76.94	48.90	38.85	6.57	50.89	41.59
	Mean	39.38	4.32	1.61	1.58	11.42	22.09	18.36	7.87	26.51	39.08	46.10	75.77	45.45	40.45	7.18	48.54	43.15
	SD	3.35	1.32	0.61	0.30	2.33	1.87	1.36	1.12	4.19	4.95	2.93	3.01	2.27	2.70	1.97	2.89	2.64

ALA=α-linolenic acid; ER=Elongation ratio; DR=Desaturation ratio; ODR=Oleic desaturation ratio; LDR=Linoleic desaturation ratio; EFA=Essential fatty acid; SFA=Saturated fatty acid; MUFA=Monounsaturate fatty acid; PUFA=Polyunsaturated fatty acid.

Table 3. Vector loadings and percentage explained variation by the first five principal components of phenotypic oil traits of *B. carinata.*

	Eigenvectors				
Composition	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
Palmitic	-0.17	-0.35	0.05	-0.13	0.10
Stearic	-0.12	-0.39	0.01	-0.11	0.05
Vaccinic	-0.30	0.11	-0.04	0.17	-0.12
Oleic	-0.24	-0.23	-0.35	0.20	-0.07
Linoleic	-0.29	0.19	0.24	0.22	0.16
α-Linolenic	-0.12	0.31	-0.41	-0.23	-0.10
Eicosenoic	0.17	0.07	-0.27	0.70	0.12
Erucic	0.34	0.08	0.19	-0.13	-0.05
ER	0.36	0.06	0.15	-0.01	-0.01
DR	-0.32	0.19	0.04	-0.12	0.08
ODR	0.14	0.34	0.32	-0.20	0.07
LDR	0.17	0.11	-0.57	-0.37	-0.24
EFA	-0.26	0.30	-0.04	0.03	0.06
SFA	-0.15	-0.37	0.05	-0.15	0.07
MUFA	0.35	-0.03	-0.09	0.25	-0.09
PUFA	-0.24	0.32	0.02	0.01	0.06
Oil	0.14	0.03	-0.27	-0.13	0.91
Eigenvalue	**6.67**	**5.26**	**1.75**	**1.57**	**1.04**
Individual percentage	**39.28**	**30.97**	**10.34**	**9.28**	**6.14**
Cumulative percentage	**39.28**	**70.25**	**80.60**	**89.87**	**96.01**

ER=Elongation ratio; DR=Desaturation ratio; ODR=Oleic desaturation ratio; LDR=Linoleic desaturation ratio; EFA=Essential fatty acid; SFA=Saturated fatty acid; MUFA=Monounsaturate fatty acid; PUFA=Polyunsaturated fatty acid.

sampling in subsequent studies and parental selection in breeding programs.

RESULTS AND DISCUSSION

Variability in the composition of the oil

The major fatty acid composition and different fatty acid ratios, of *B. carinata* oil, i.e. palmitic, stearic, oleic, vaccinic, linoleic, α-linolenic (ALA), eicosenoic, erucic, elongation ratio (ER), desaturation ratio (DR), oleic desaturation ratio (ODR), linoleic desaturation ratio (LDR), essential fatty acid (EFA), saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA) and oil content are displayed in Table 2. Oil content varied from 25 to 48%. In all accessions, the predominant fatty acids were erucic acid (6.91-35.05%), linoleic acid (17.21-28.53%), α-linolenic acid (10.50-22.52%), oleic acid (8.91-24.64%) and eicosenoic acid (1.22-10.24%). Palmetic, stearic and vaccinic were also present in small quantities.

Principal component analysis

The eigenvalues often are used to determine how many factors to retain. When the PCA is run on correlations, one rule-of-thumb is to retain those factors whose eigenvalues are greater than one. The sum of the eigenvalues is equal to the number of variables. Hence, in this analysis the first factor retains the information contained in 6.67 of the original variables. The coefficients defining the five principal components of these data are given in Table 3. These coefficients are scaled, so that they present correlations between observed variables and derived components. This analysis suggests that the first five principal component scores for each individual might act as an adequate summary of the original 17 variables in any further analysis of the data; the first five components extracted from the original data had latent roots greater than one, accounting for nearly 96.01% of the total variation of the original variables. These were thus retained as grouping variables in the subsequent cluster analysis.

The first principal component accounted for 39.28% of the total variance, the second a further 30.97%, the third 10.34%, the fourth 9.28% and the fifth 6.14% of the total variance. The components can be interpreted in terms of the variables, which loads “most heavily” on to them (i.e. have the highest component loadings).

The first component had high positive loadings from ER, MUFA, and erucic acid and high negative loadings from DR, vaccinic acid, and EFA. This component is then

Table 4. Mean and standard deviation of oil content and fatty acid composition in the classification of clusters.

Cluster	Oil (%)	Palmitic (16:0)	Stearic (18:0)	Vaccinic (18:1c7)	Oleic (18:1c9)	Linoleic (18:2c9,12	ALA (18:3c9,12,15)	Eicosenoic (20:1c11)	Erucic (22:1c13)	ER (%)	DR (%)	ODR (%)	LDR (%)	EFA (%)	SFA (%)	MUFA (%)	PUFA (%)
I	28.83	4.16	1.43	1.51	11.65	21.66	18.20	7.99	27.65	0.40	0.45	0.75	0.46	39.85	6.77	49.96	42.49
	2.89	0.04	0.10	0.60	0.81	0.78	0.34	0.83	0.80	0.01	0.01	0.02	0.04	1.12	0.24	0.86	1.19
II	40.59	3.86	1.52	1.39	11.50	20.25	18.29	8.47	28.66	0.42	0.44	0.75	0.48	38.54	6.68	51.15	41.18
	2.31	0.23	0.11	0.18	1.57	0.86	0.73	0.44	1.91	0.02	0.01	0.02	0.02	0.85	0.32	1.20	0.81
III	39.66	4.25	1.54	1.60	10.78	22.53	18.52	7.81	26.75	0.39	0.47	0.77	0.45	41.05	7..03	48.10	43.86
	2.51	0.32	0.15	0.15	1.05	1.06	0.90	0.63	2.09	0.02	0.01	0.01	0.02	1.06	0.60	1.52	0.97
IV	29.95	5.06	1.86	2.98	20.66	28.52	22.36	5.41	7.82	0.15	0.58	0.68	0.44	50.88	8.04	37.71	52.23
	7.11	0.60	0.13	0.17	1.30	0.02	0.22	0.71	1.29	0.02	0.05	0.01	0.02	0.24	0.40	0.54	0.05
V	41.15	5.79	1.90	2.66	16.36	26.55	19.36	8.74	13.32	0.25	0.53	0.71	0.42	45.91	8.77	42.01	47.80
VI	38.12	6.21	1.44	1.94	11.65	25.34	19.66	6.72	20.00	0.31	0.53	0.77	0.44	45.00	8.75	41.50	47.38
VII	39.90	3.63	1.64	1.28	10.48	20.56	16.87	1.22	30.51	0.39	0.46	0.76	0.45	37.43	6.62	44.70	40.29
VIII	33.14	3.53	1.63	1.33	12.23	19.43	16.68	10.24	29.38	0.44	0.40	0.73	0.46	36.11	6.59	54.16	38.52
IX	35.07	16.42	7.43	1.28	24.64	17.21	10.50	4.36	11.59	0.23	0.40	0.52	0.38	27.70	25.55	43.47	29.18
X	41.30	4.25	1.57	1.69	13.91	24.38	16.01	10.15	22.59	0.36	0.46	0.72	0.40	40.40	6.95	49.27	42.73
XI	43.50	3.38	1.32	1.30	10.31	17.38	15.31	9.13	35.01	0.50	0.37	0.74	0.47	32.68	6.06	57.05	35.72

ALA=α-linolenic acid; ER=Elongation ratio; DR=Desaturation ratio; ODR=Oleic desaturation ratio; LDR=Linoleic desaturation ratio; EFA=Essential fatty acid; SFA=Saturated fatty acid; MUFA=Monounsaturate fatty acid; PUFA=Polyunsaturated fatty acid.

Table 5. Euclidean genetic distance ranges for Ethiopian mustard (*B. carinata*) accessions.

Genetic distance measure (range)	Frequency (%)
<0.30	3
0.31-0.40	5
0.41-0.50	8
0.51-0.60	10
0.61-0.70	11
0.71-0.80	11
0.81-0.90	10
0.91-1.00	8
1.01-2.00	26
2.01-3.00	3
>3.01	5

describing the general trend of correlations resulting from the higher rate of increase of ER, the lower increase of MUFA and erucic acid, the highest rate of decrease of DR and the lowest rate of decrease of vaccinic and EFA. What a positive loading indicates, is that there is a positive correlation between the component and the variable.

The second component, accounting for 30.97% of the total variance, had high positive loadings from ODR, PUFA, α-Linolenic acid and EFA, and high negative loadings from stearic, SFA, and palmitic acid. The component is used to describe the trends in correlations resulting from the highest rate of increase in ODR, and highest rates of decrease in stearic acid, SFA and palmitic acid.

The third component, explaining 10.34 % of the total variance, had high positive loadings from ODR, linoleic, and erucic acids and high negative loadings from LDR, α-Linolenic, and oleic acids.

Five groups were identified by reducing the number of variables. It can be argued that only one variable from each should be studied. For the first group, ER would seem to be the best choice, it has the largest loading from component one, whereas stearic would be the best in the second group, LDR for the third, eicosenoic acid for the fourth, and oil content for the fifth. Hence, the 17 original variables, among which there was substantial inter-correlation, have been replaced by five, which are weighted combinations of the former and are independent of each other. Figure 2 presents the distribution in space of *B. carinata* accessions in relation to the first three principal components.

Estimates of genetic distance

The data matrix of oil content, fatty acid composition and fatty acid ratios formed the basis of Euclidean genetic distance calculations for all 4753 pair wise comparisons of the 98 genotypes (ranges shown in Table 5). In this study a wide range of genetic distances was observed among genotypes. The estimates of Euclidean genetic distance value ranged from 0.16 (between 'PGRC/E 21263' and 'PGRC/E 20080') to 6.04 (between 'PGRC/E 20104' and 'C94-S-67'). The mean genetic distance between all pairs of comparisons was 1.08 ± 0.02. The frequency distribution of genetic distance values for all 4753 pairs of comparisons indicated that 76% of the pair wise comparisons had values between 0.51-2.00. Less than 3% of the pair wise comparisons had a genetic distance value of smaller than 0.30 and more than five percent were larger than 3.01. Of this group of genotypes, 'C94-S-67' and 'C94-Dodolla' had the greatest genetic distance from all other genotypes in this experiment. The range in Euclidean genetic distances from 0.16 to 6.04 demonstrates the diversity in this germplasm. Estimates of genetic distance values show that there is a wide range of variation among the Ethiopian mustard genotypes. This information can be used to plan crosses, to exploit genetic diversity and to maximize the expression of heterosis.

Table 6. Clustering pattern of 98 Ethiopian mustard (*B. carinata*) genotypes.

Cluster	No. of genotypes	Genotypes in each cluster*
I	2	30, 63
II	22	7,12,15,16,18,19,23,35,36,37,45,51, 54,56,57,60,64,78,79,94,96,98
III	65	1,2,3,8,9,10,11,12,13,14,17,20,21,22 ,24,25,26,27,28,29,31,32,33,38 39,40,41,42,43,44,46,48,49,50,52,53 ,59,61,62,65,66,67,68,69,70,71, 72,73,74,75,77,81,83,84,85,86,87,88 ,89,90,91,92,93,95,97
IV	2	5,6
V	1	4
VI	1	47
VII	1	55
VIII	1	58
IX	1	76
X	1	80
XI	1	82

*See table 1 for the number references

Cluster analysis

The clusters from the UPGMA clustering method of the 98 Ethiopian mustard genotypes is depicted in Table 6. Mean and standard deviation of the quantitative phenotypic oil traits for each cluster are presented in Table 4. These are descriptive statistics once the clusters are given. It is tempting to judge differences between clusters with traditional t-test. However, these tests are invalid in cluster analysis context. Moreover, the degree of (absence of) overlap of clusters is more relevant than statistical significance of a systematic difference.

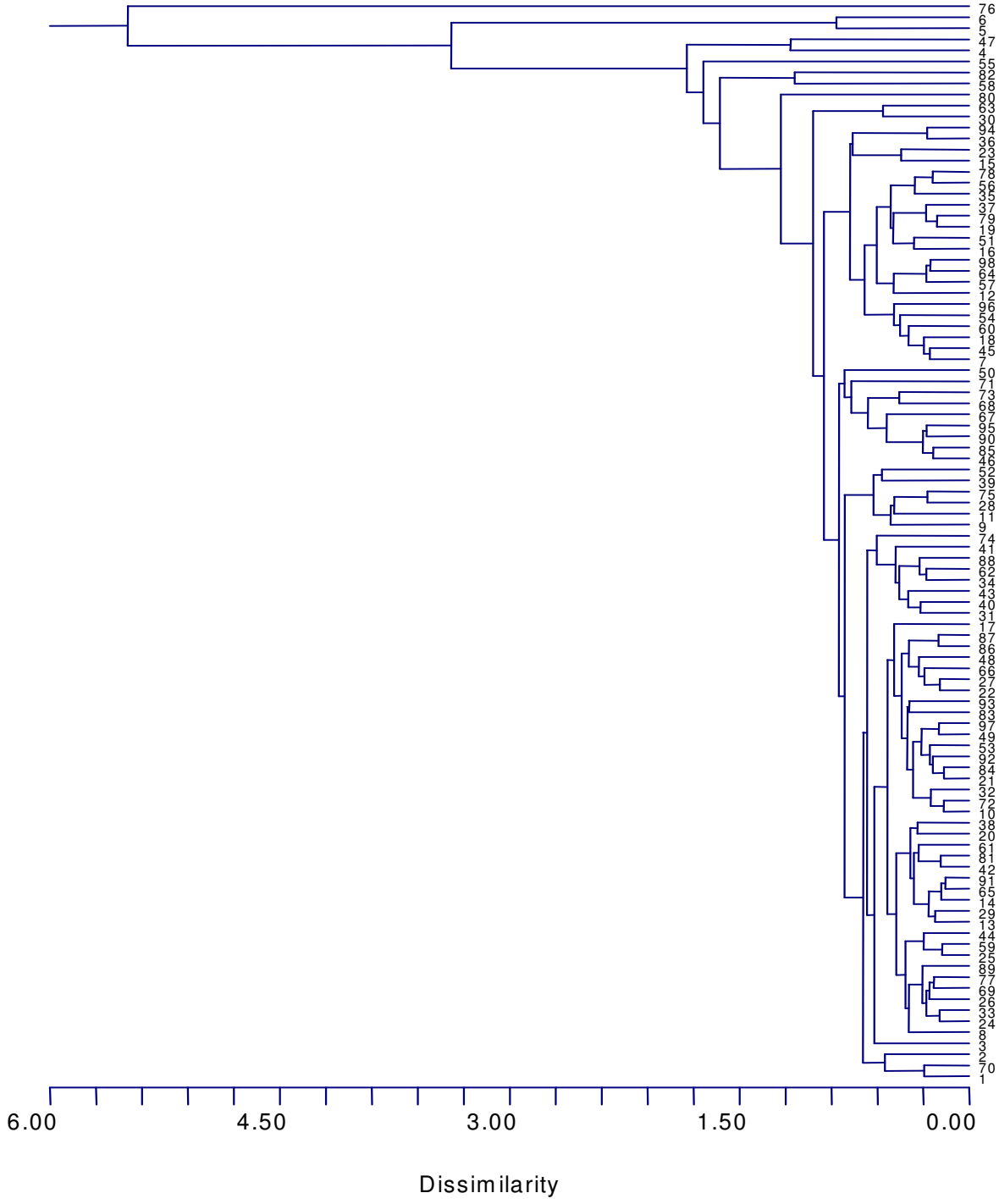

Figure 1. Dendrogram generated based on UPGMA clustering method depicting genetic relationships among 98 *B. carinata* genotypes based on fatty acid composition data.

Therefore, results will be discussed here rather informally.

At a cutoff 0.90 the denderogram revealed 11 distinct clusters (Figure 1). Four are real clusters and seven singletons. These singletons are also called clusters even though they consist of only one genotype. A good fit with the genetic distance matrix values could be confirmed by a cophenetic correlation coefficient of r=0.96. Cophenetic values of 0.75 or more are usually recommended for the best fit of the cluster analysis. The two delta goodness of

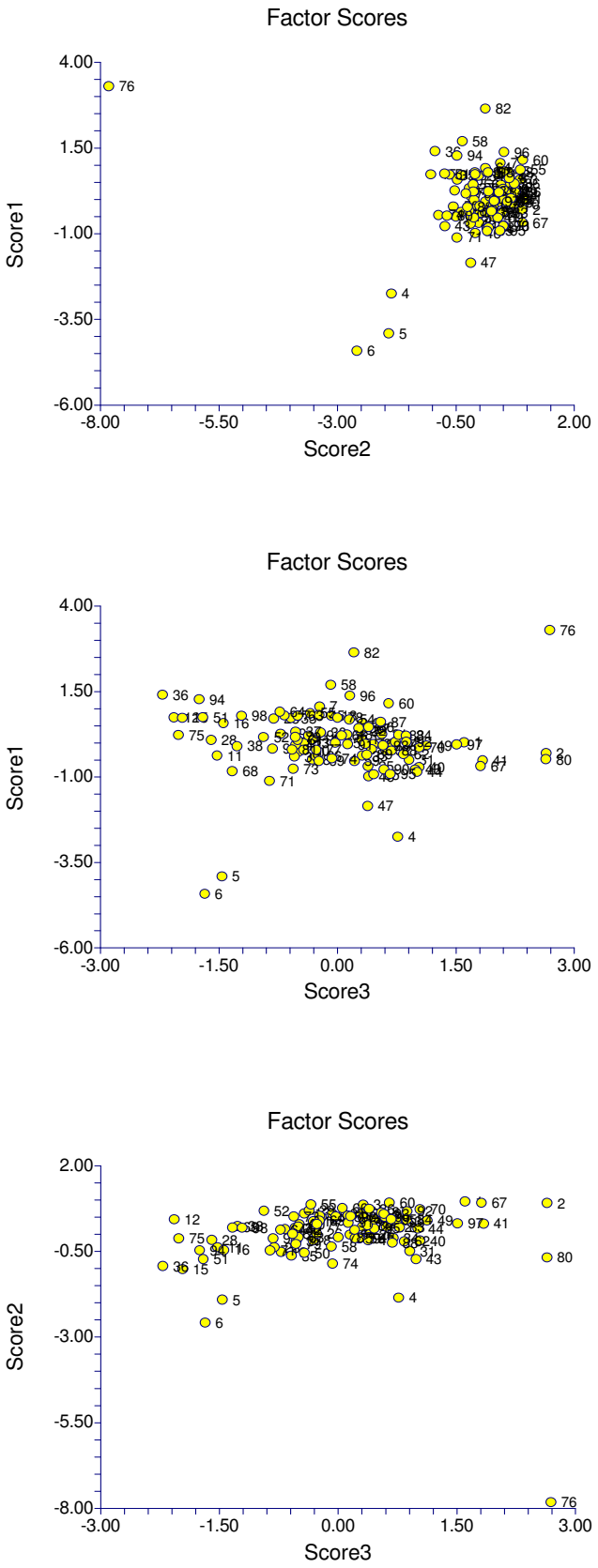

Figure 2. The distribution of Ethiopian mustard (*B. carinata)* accessions in relation to the first three principal components.

fit statistics delta (0.5) and delta (1.0) were 0.179 and 0.190, respectively. When comparing to clustering configurations, the configuration with the smallest delta value fits the data better.

Cluster I was mainly characterized by low oil content. It contained two accessions 'PGRC/E 20147/1' and 'PGRC/E 21162' with a genetic distance value of 0.58. Cluster II contained 22 accessions and was mainly characterized by high oil content and LDR. The range of dissimilarities obtained was 0.21 (between 'PGRC/E 21356/1' and 'PGRC/E 207931') to 1.3 (between 'PGRC/E 207929' and 'PGRC/E 21224/2'). The mean genetic distance value of all accessions was 0.65 ± 0.02. Cluster III contained the largest number of accessions. Estimates of genetic distance values range from 0.16 (between 'PGRC/E 20080' and 'PGRC/E 21263') to 3.00 (between 'PGRC/E 210406' and 'PGRC/E 21261'). The mean genetic distance value of all accessions in this cluster was 0.68 ± 0.01. Cluster IV had two accessions from Canada, 'C94-S-67' and 'C94-Dodolla', with a genetic distance of 0.87 and were characterized by low oil content, erucic acid, ER, and MUFA and high vaccinic, oleic, linoleic, and α-Linolenic acids, DR, LDR, EFA and PUFA. Cluster V had only one accession with high stearic, α-linolenic, and eicosenoic acids. Cluster VI contained only one accession with high palmitic and α-linolenic acids. Cluster VII with only one accession had high ODR and erucic acid. Cluster VIII contained only one accession with high eicosenoic acid and low oil content. Cluster IX had only one accession with high palimitic, stearic, oleic acids and SFA content and low PUFA. Cluster X with only one accession, had high oil content and linoleic acid. Cluster XI with only one accession had high erucic acid, oil content and MUFA. Divergent genotypes may have good breeding values. Genotypes in the same cluster may represent members of one heterotic group. Maximum variability for selection in segregating population may be achieved by utilizing genotypes from different clusters as parents of crosses.

Dissimilarities obtained in this study show a wide variation in land races of *B. carinata*. It is probable that greater diversity would be seen if a wider collection from more distant geographical regions was included. However, the knowledge of genetic relationships generated from this investigation will be of value in the exploitation of available germplasm.

CONCLUSION

In conclusion this study revealed wide variation in fatty acid composition. Principal component analysis revealed that DR, ER, MUFA, ODR, and vaccinic acid had the highest loading in the first component that accounted for 39.28% of the total variation. In the second principal component, stearic acid, SFA, palmitic acid, ODR, PUFA, and α-linolenic acid had the highest loading that

accounted for 30.97% of the total variation. Genetic distance estimates based on fatty acid composition revealed that the Ethiopian mustard genotypes had a fairly high mean genetic distance value. The dendrogram generated by the UPGMA cluster analysis based on Euclidean genetic distance estimates grouped *B. carinata* genotypes into 11 distinct clusters. Therefore, the information generated from this study can be used to plan crosses and maximize the use of genetic diversity and heterosis.

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