

¹Holetta Research Centre, Ethiopian Agricultural Research Organization, Addis Ababa, Ethiopia

²Georg-August University, Institute of Agronomy and Plant Breeding, Germany

Variation and covariation of seed quality traits in Ethiopian mustard

Adefris Teklewold^{1,2}, Heiko C. Becker²

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Summary

Ethiopian mustard (*Brassica carinata* A. Braun), an oilseed crop of Ethiopian origin, is a less preferred source of edible oil due to its high erucic acid and glucosinolate contents. This study was undertaken to assess the variability for seed quality traits in the Ethiopian germplasm, determine their interrelationships and explore their pattern of genetic variation with respect to geographic origin. Seed of 913 selfed S₂ plants derived from 36 germplasm accessions collected from eight different geographic regions of Ethiopia were analysed for seven seed quality traits by Near Infrared Reflectance Spectroscopy (NIRS). Large variability for oil, protein and glucosinolate contents was observed ranging from 16.4 to 54.7 %, 19.0 to 39.3 % and 28.2 to 171.8 µmoles g⁻¹, respectively. Fatty acids varied as follow: Oleic, 0 to 20.3 %; linoleic, 5.4 to 29.5 %; linolenic, 10.1 to 22.9 %; and erucic acid 14.3 to 53.3 %. Correlation analysis showed a strong negative associations between oil and protein contents and oleic and erucic acids. Mahalanobis' dissimilarity index showed high diversity and the 913 S₂ plants were grouped by the unweighted pair-group method using arithmetic mean (UPGMA) into 11 clusters, that varied in constellation from a cluster that contain a single plant to a cluster containing 332 plants. Factors other than geographic origin appeared to be a potent source of genetic diversity. The result demonstrated the usefulness of assessing genetic variation in segregating progenies to detect rare but desired traits like low glucosinolate content.

Introduction

The value and usefulness of vegetable oil is determined by its fatty acid composition and other seed oil quality parameters (MCVETTY and SCARTH, 2002). Despite its long history of cultivation in Ethiopia (SIMMONDS, 1979), the use of Ethiopian mustard (*Brassica carinata* A. Braun) as source of edible oil has been limited because the oil is characterized by high level of erucic (> 40%) and linolenic acids (BECKER et al., 1999). The meal obtained after oil extraction contains about 39 % crude protein (SEYOUN, 1995). However, it is not preferred as feed for animals due to the presence of high amount of glucosinolate. In Canada and many West Europe countries, quality breeding played a major role in making rapeseed (*B. napus*) a dominant source of vegetable oil and oilseed protein. The preference of the crop have been greatly enhanced after modification of the fatty acid and glucosinolate profiles to suit human and animal nutritional requirements (RÖBBELEN and THIES, 1980b; BECKER et al., 1999). In improving *B. carinata* as nutritionally enhanced oilseed crop in Ethiopia and elsewhere, considerable efforts are now being directed towards improving the fatty acid composition profile in the oil and the glucosinolate content in the meal (RAKOW, 1995; GETINET et al., 1996; VELASCO et al., 1998; ALEMAYEHU and BECKER, 2002).

Analysis of genetic diversity in germplasm collections can facilitate reliable classification of accessions and identification of subset core accessions with possible utility for specific breeding purpose (MOHAMMADI and PRASANNA, 2003). Moreover, to make further

productive gains in the long term, new sources of variation need to be explored and utilized. Accordingly, GETINET et al. (1996) and ALEMAYEHU and BECKER (2001, 2002) have studied the diversity of seed quality traits in Ethiopian germplasm. They reported large variation for some seed quality traits, but variability necessary to meet most quality requisites that can make *B. carinata* nutritionally acceptable oil crop are still lacking.

Genetic diversity arises either due to geographic separation or genetic barrier to crossability (SINGH, 1996). According to LINHART and GRANT (1996) and RAO and HODGKIN (2002), natural selection acting on heritable phenotypic variation will result in adaptation and differentiation among populations of the same species inhabiting environments differing in their selective regime. Therefore, information about the structure of the genetic diversity and the relationship of diversity with geographic origin will be essential to exploit traits of economic importance.

B. carinata is believed to possess substantial rate of outcrossing. Therefore, the level of diversity reported from accessions is an average effect and may not reflect the complete level of genetic variation in the germplasm as the accessions are heterozygous and heterogeneous. Rare but useful alleles may not be detected when analysing the accessions themselves. A detailed insight in such variability studies require analysing the variation of either individual plants or segregating progenies of the accessions.

The present study was undertaken to assess the extent of variability present within the Ethiopian germplasm for important seed quality traits, determine their interrelationships and explore their pattern of genetic variation with respect to geographic origin. For this purpose, 913 S₂ plants that were derived from 36 germplasm accessions collected from eight different geographic regions in Ethiopia were used.

Materials and methods

Source materials for this study were 36 *B. carinata* accessions representing eight *B. carinata* growing areas in Ethiopia (Tab. 1). During June - December 1999, seed from the 36 germplasm collections were planted at Holetta, Ethiopia. Holetta is located 45 km west of Addis Ababa (the capital city of Ethiopia) and has an altitude of 2400 m a.s.l. The long-term average annual rainfall is about 1095 mm and the daily mean temperature ranges between 2 and 22 °C. The 36 accessions were planted in unreplicated plots of 2.4 m², each plot having four rows of 30 cm inter-row spacing and 2 m length. In each row, 41 individual plants were grown at 5 cm spacing. Crop management factors like land preparation, rotation schemes, fertilizer and weed control were carried out according to the local recommendation. Before flowering, eight to ten single plants were chosen randomly from each plot and were selfed. In 2000, 259 rows of S₁ plants were grown in off-season-field under irrigation. From each row, 5 random plants were selfed. In 2000/2001, 1085

rows of S_2 plants were grown and progenies derived from each accession were grouped phenotypically into uniform groups. Then one progeny was randomly chosen from each uniform group to constitute in total 208 progenies. From each of the 208 progeny rows, five plants were randomly chosen and selfed. The seed from these 1040 selfed single S_2 plants was harvested separately.

Of the 1040 single S_2 plants, only 913 produced good and sufficient seeds for Near Infrared Reflectance Spectroscopy (NIRS) analysis. Three gram of the S_3 seed was scanned by NIRS (a monochromator model 6500, NIR System, Inc., Silver Springs, MD) to determine oil, protein and total glucosinolate contents and fatty acid composition on percentage basis. The multitrait calibration equation developed for *B. carinata* by VELASCO et al. (1995a) was used to convert the spectral data into quantitative values. Generalized Mahalanobis' squared distance (D^2) statistics was calculated from the NIRS data for all possible pairs of 913 S_2 plants. The distance matrix was used to group the S_2 plants following unweighted pair-group method using arithmetic mean (UPGMA). Guided by the pseudo F and t^2 statistics, 11 clusters were taken as acceptable number of clusters. Mahalanobis

squared distance (D^2) matrix was calculated from the mean value of members in each of the 11 clusters and used to construct dendrogram. The multivariate statistical analyses were done by SAS software (SAS, 2002).

Results

A wide range of variability was observed for oil content, ranging from 16.4 % to 54.7 % with a mean of 32.3 % (Tab. 2). Referring to the frequency distribution (Fig. 1), 17 plants exhibited oil content of greater than 46 % and three of these had oil content more than 52 %. Mean protein content was 30.1 % and ranged from 19.0 to 39.0 %. Ninety two plants had protein contents greater than 34.0 % of which five plants had more than 37.0 %. The average crude protein content in the meal was calculated to be around 44 %. Glucosinolate concentration in the whole seed ranged from 28.2 to 171.8 $\mu\text{moles g}^{-1}$, with a mean of 108.1 $\mu\text{moles g}^{-1}$. Plants falling towards the extreme low tail of the distribution were relatively rare as compared to the extreme high tail such that only five plants represented the lowest

Tab. 1: Accessions used to develop the S_2 plants for studying variability of seed quality traits in *B. carinata* and their area and altitude of collection

Acc. No [†]	Area of collection	Altitude (m)	Acc. No	Area of collection	Altitude (m)	Acc. No	Area of collection	Altitude (m)
21194	Welega	1980	21005	Arssi	2450	21246	Gonder	1970
20068	Shewa	2010	21209	Welega	2460	21193	Welega	2480
21278	Wolo	2290	20052	Shewa	2800	21002	Arssi	2350
21369	Kefa	1772	21176	Welega	2180	21156	Welega	2480
21245	Gonder	1860	21224	Kefa	1750	21316	Shewa	2430
21080	Arssi	2400	208404	Gojam	1960	21276	Wolo	2290
21068	Bale	2500	21005	Arssi	2450	207486	Gonder	2350
21255	Gojam	2000	21071	Bale	2640	21007	Arssi	2900
21163	Welega	2340	21182	Welega	2120	21236	Gojam	2350
21248	Gonder	1890	21380	Shewa	1640	21192	Welega	2090
21069	Bale	2450	21253	Gojam	1740	21374	Shewa	1220
21266	Wolo	2570	21265	Wolo	1950	21289	Wolo	2570

[†]Accession code refers the accession identification number of the Institute of Biodiversity Conservation, Ethiopia

Tab. 2: Variation in seven seed quality traits of the 913 S_2 plants of Ethiopian mustard

Seed quality traits	Minimum	Maximum	Mean	Standard deviation	Coefficient of variation
Oil	16.4	54.7	32.3	6.1	18.9
Protein	19.0	39.3	30.1	3.3	10.8
Glucosinolate	28.2	171.8	108.1	22.3	20.7
Oleic acid	0.0	20.3	7.2	3.6	49.6
Linoleic acid	5.4	29.5	19.5	3.1	16.1
Linolenic acid	10.1	22.9	16.9	2.1	12.4
Erucic acid	14.3	53.3	33.4	4.4	13.2

Oil and protein contents expressed in % of the intact dried seed; fatty acid in % of the total fatty acids; glucosinolate content in $\mu\text{mol g}^{-1}$; coefficient of variation in %.

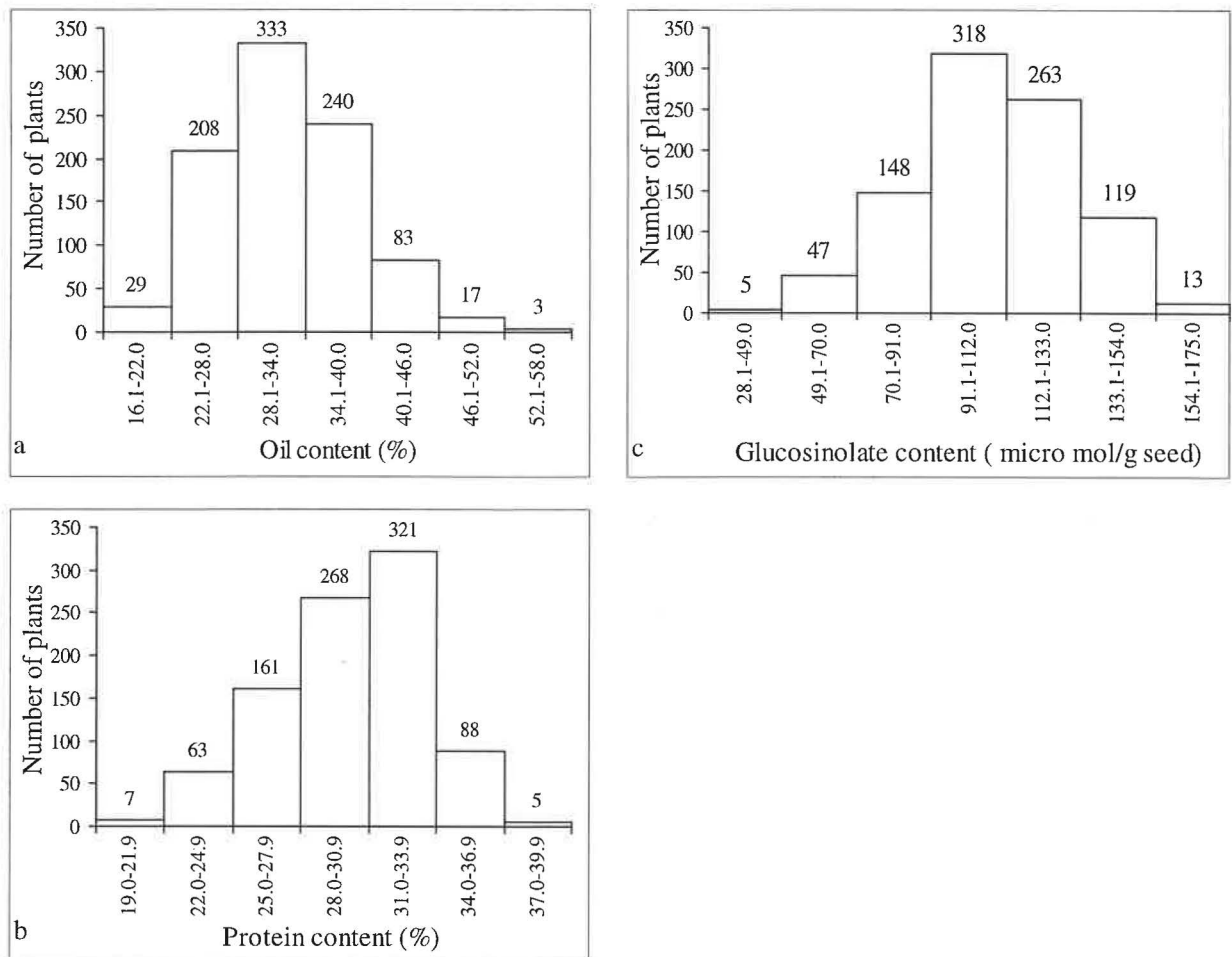


Fig. 1: Frequency distribution of (a) oil, (b) protein and (c) glucosinolate contents for the 913 S_2 plants

glucosinolate content class (28 to 49 $\mu\text{moles g}^{-1}$), while 13 plants represented the highest glucosinolate class (154 to 175 $\mu\text{moles g}^{-1}$).

Regarding the fatty acid composition erucic acid constituted the major proportion followed by linoleic, linolenic and oleic acid (Tab. 2). On average, erucic, linoleic, linolenic and oleic acids constituted 33.4 %, 19.5 %, 16.9 % and 7.2 % of the total fatty acids in the oil, respectively. Except linolenic acid, the other three fatty acid had large variability as measured by their amplitude: 20.3 %, 24.1 % and 39.0 % for oleic, linoleic, and erucic acid, respectively. The amplitude for linolenic acid was only 12.8 %. As shown in Fig. 2, the frequency distribution of oleic acid was biased towards low oleic types. About 70 % of the plants (635 plants) contained oleic acid less than 9.0 %. The frequency distribution with respect to linoleic acid was the converse of oleic acid. About 89 % of the plants had linoleic acid more than 15.9 %. The distribution of linolenic acid seems more symmetrical. Eighty-one plants had less than 14.1 %, while 57 had greater than 20.1 % linolenic acid, the remaining plants fell in-between. The frequency distribution of erucic acid depicts the scarceness of low erucic acid genes in Ethiopian germplasm pool. Only four plants showed below 20.0 % erucic acid content, of which one had 14.3 %. Among the seven seed quality traits, oleic acid showed the highest coefficient of variation of 49.6 % followed by glucosinolate (20.7 %).

Phenotypic correlation coefficients were calculated to determine the relationship among fatty acids and oil content (Tab. 3) and between

oil and protein contents (Fig. 3). All correlation coefficients were statistically significant. Nevertheless, there is a marked difference in their direction and magnitude varying from 0.19 between oil and linolenic acid contents to -0.72 between oil and protein contents. Linolenic and erucic acid were negatively correlated to oleic and linoleic acids but positively among themselves and to oil content. Oleic and linoleic acids were negatively correlated to oil content, while they were positively correlated to each other.

In grouping the plants into clusters, 11 clusters were taken as optimal number of clusters (Tab. 4). Cluster 3, the largest of all, was formed by 332 plants derived from accessions that were collected from all the eight geographic areas of the country. Clusters 1, 2, 4 and 6 were the next biggest clusters containing 217, 165, 104 and 64 plants, respectively. Each of these four clusters contained plants derived from accessions that were collected from all the eight geographic areas of the country. Twenty plants derived from accessions that were collected from five geographic areas constituted cluster 5. In cluster 7, six plants derived from three accessions of different geographic origin were included. Cluster 8 contained two plants each derived from different accessions with different geographic origin. The remaining three clusters (clusters 9, 10 and 11) were solitary-entry-clusters. The inter-cluster distances varied from 7.73 (between cluster 4 and 6) to 366.72 (between cluster 5 and 7).

Clustering the plants in to relatively homogeneous group enabled to clearly mark those that excel in their mean performance for the

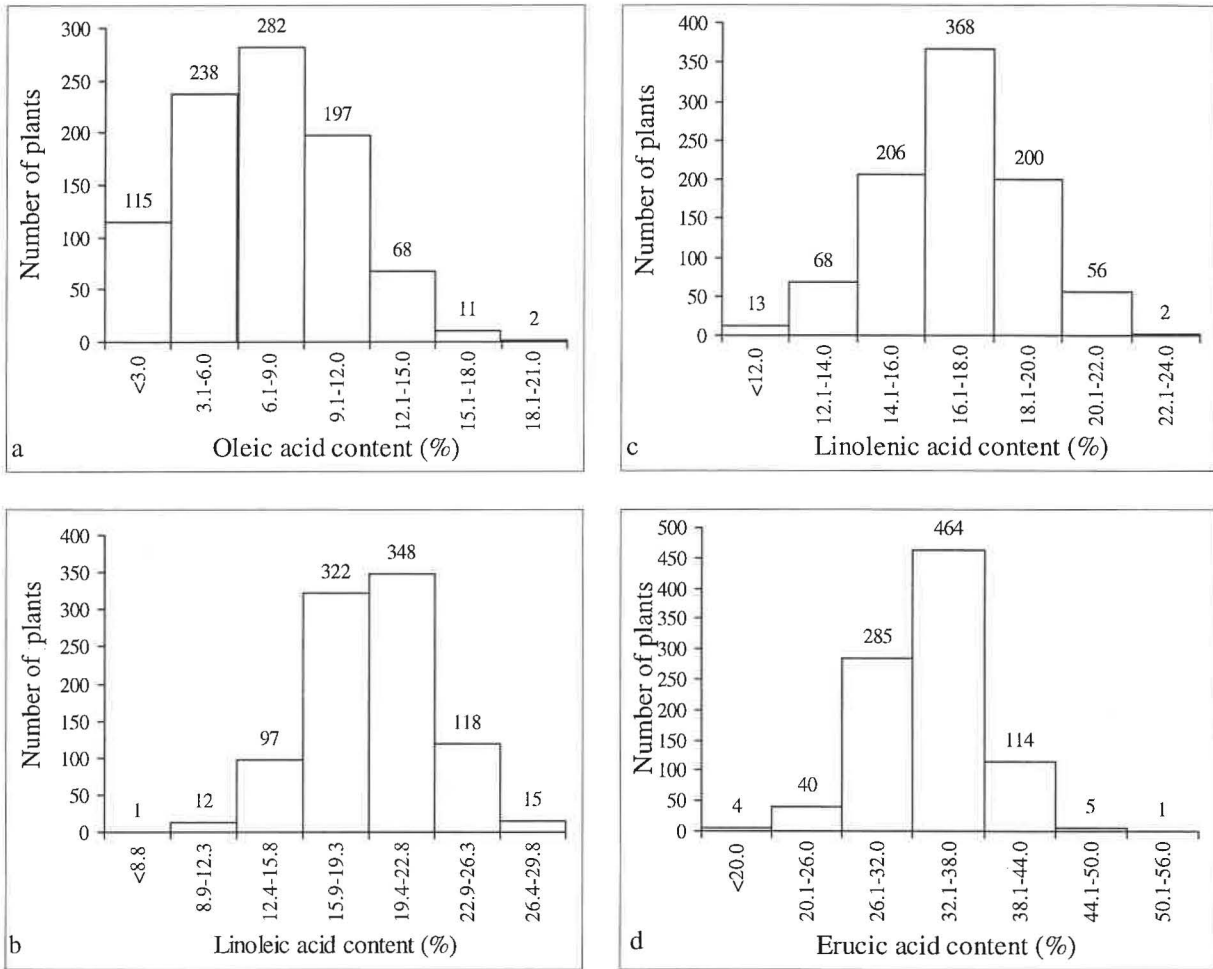


Fig. 2: Frequency distribution of (a) oleic, (b) linoleic, (c) linolenic and (d) erucic acids for the 913 S₂ plants

Tab. 3: Correlation coefficient among fatty acids and oil content

Quality traits	Oleic acid	Linoleic acid	Linolenic acid	Erucic acid	Oil
Oleic acid	1	0.22**	- 0.40**	- 0.54**	- 0.23**
Linoleic acid		1	-0.51**	-0.69**	-0.51**
Linolenic acid			1	0.24**	0.19**
Erucic acid				1	0.54**
Oil					1

** indicate significance at P ≤ 0.01.

particular seed quality traits (Tab. 4). Mean linoleic acid concentration was highest for cluster 11 and lowest for cluster 7. Lowest mean of linolenic acid was exhibited by the plants in cluster 9; the highest was recorded from cluster 4. Highest erucic acid content was from cluster 6, while cluster 11 had the lowest. Cluster 7 contained plants with highest mean oil content, while cluster 8 contained the lowest. The lowest mean protein content was recorded from cluster 9 and the highest from cluster 8. Highest mean glucosinolate was obtained from cluster 5, while the lowest from cluster 7. The relationship between the 11 clusters based on Mahalanobis' distance index is shown in a dendrogram (Fig. 4). The dendrogram

has divided the 913 plants into two distinct groups at about 1.2 dissimilarity index, still these two major groups dispersed into distinct sub-groups. Of course, considering their dissimilarity index, all the 11 clusters could be taken each as a distinct group as the two most similar clusters (Cluster 4 and 6) were linked on the dendrogram at about 0.3 dissimilarity index. The formation of the two major groups in the dendrogram seems largely influenced by glucosinolate content. Clusters 1, 2, 3 and 11 that contained plants with relatively higher amount of glucosinolate were sorted under one major group while the other clusters with relatively lower level of glucosinolate formed a distinct group.

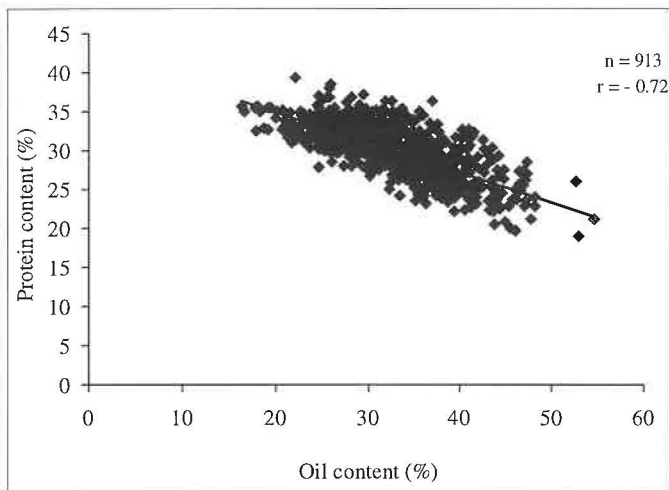


Fig. 3: Association of oil and protein contents

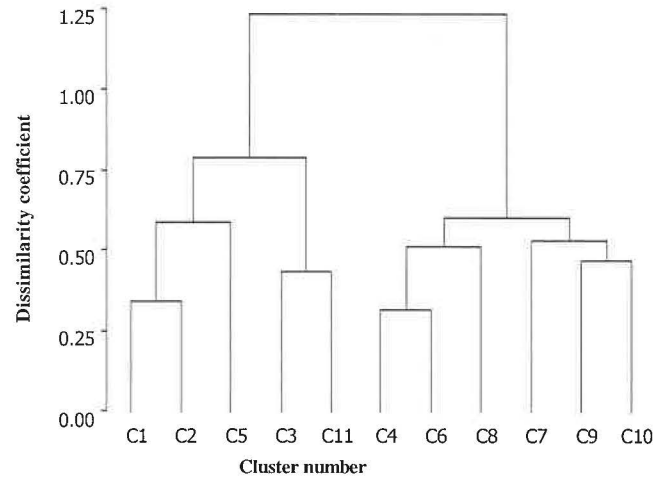


Fig. 4: UPGMA based dendrogram of 11 clusters formed by the 913 S_2 plants Mahalanobis' distance matrix (on the basis of mean values of the members included in each cluster)

Tab. 4: Cluster means for the seven seed quality traits of the 913 S_2 plants of *B. carinata*

Cluster no	C 18:1*	C 18:2	C 18:3	C22:1	Oil	Protein	GSL	No of plants [†]	Collection area [‡]
1	7.17	19.93	16.72	33.48	31.85	30.85	119.12	217	1-8 §
2	7.39	21.21	16.53	31.98	28.78	32.70	136.57	165	1-8
3	7.21	19.28	17.02	33.45	32.65	29.66	100.93	332	1-8
4	6.95	17.58	17.53	34.75	35.65	27.98	83.72	104	1-8
5	7.38	22.18	15.59	31.25	28.37	32.47	157.00	20	1,2,4,6,7,8
6	6.47	17.40	17.35	35.51	37.34	26.38	67.35	64	1-8
7	8.17	17.08	16.60	35.28	38.69	26.25	44.69	6	1,6,7
8	6.83	25.80	16.38	24.24	17.43	35.33	80.60	2	2
9	20.28	22.80	13.17	19.75	37.84	24.80	66.18	1	1
10	9.61	22.91	17.14	22.24	21.61	32.69	54.57	1	1
11	9.62	29.51	14.56	14.29	32.49	25.88	104.27	1	1

* C 18:1= oleic acid, C 18:2= linoleic acid, C 18:3= linolenic acid, C22:1 = erucic acid and GSL= glucosinolate

[†] Number of plants included in the respective cluster

[‡] Geographic area from where the parental accessions were collected

§ Numerical code of geographic area from where the parental accessions were collected (1 = Arssi, 2 = Bale, 3 = Gojam, 4 = Gonder, 5 = Kefa, 6 = Shewa, 7 = Welega and 8 = Wolo)

Discussion

As an oil crop, the value of *B. carinata* lies more in its seed quality traits than any other plant traits. In this regard, this study depicted large amounts of variability for oil content ranging from 16.4 % to 54.7 % with a mean of 32.3 %. The frequency distribution for oil content, however, indicates that genotypes with high oil content are rare and thus selecting for high oil content would be relatively difficult. The range of oil content obtained in this study is much higher than reported earlier. GOERING et al. (1965) (cited in RÖBBELEN and THIES, 1980a) reported a range of 29.4 to 38.1 % and ALEMAYEHU and BECKER (1999) reported 20 to 41 % oil content in germplasm materials of *B. carinata*.

The average crude protein content of the seed (30.1 %), which is calculated to be about 44 % in the meal, suggests that the meal from *B. carinata* has protein content comparable to that of *B. napus*. The average protein content in *B. napus* was reported to be around 38 % (BECKER et al., 1999). ALEMAYEHU and BECKER (1999) also reported protein content that ranged from 29 to 45 % in *B. carinata*. The seed cake, hence, could be a useful protein supplement provided that the glucosinolate content is reduced to the level that does not cause pungency and health hazard to animals.

The range for glucosinolate content indicates the availability of wide natural variability with both low and high contents; as low as

28.2 $\mu\text{moles g}^{-1}$ and as high as 171.76 $\mu\text{moles g}^{-1}$ glucosinolate content was recorded. Nevertheless, the frequency of plants with lower amount of glucosinolate was much lower than with higher glucosinolate content. This confirms the rare occurrence of low-glucosinolate genes in *B. carinata* gene pool. The mean value for glucosinolate content observed in this study is very much similar to the results reported by GETINET et al. (1996), ALEMAYEHU and BECKER (1999, 2002), but is lower than the result reported by VELASCO et al. (1995b, 1998). The minimum glucosinolate content observed in this study, however, is lower than all values reported before. The use of segregating plants as a study material might have contributed for detecting genotypes with such low level of glucosinolate content. Because *B. carinata* shows a substantial amount of outcrossing, accessions are heterogeneous and heterozygous. Hence, values measured from an accession are an average expression of different individuals. Some traits like low glucosinolate content are rare in nature and genes controlling them are difficult to identify in the accessions. Moreover, low glucosinolate content is determined by multiple recessive genes (RÜCKER and RÖBBELEN, 1994) that presuppose selection of low glucosinolate genotypes in larger number of segregating progenies.

The fatty acid composition of *B. carinata* is typical for other *Brassicacae*, erucic acid constituting the major proportion of the total fatty acid followed by linoleic, linolenic and oleic acid (RÖBBELEN and THIES, 1980a; RUDLOFF, 1995; BECKER et al., 1999). The mean linoleic acid content, 19.5 %, obtained in this study is very similar to what was reported by RÖBBELEN and THIES (1980a) and is higher as compared to the other *Brassica* oil crops (13.4 to 16.1 %). Higher concentration of linoleic acid content is desirable for nutritional reasons (RÖBBELEN and THIES, 1980a). The mean linolenic acid content observed in this study (16.9 %) was similar to the 15.9 % reported by VELASCO et al. (1998), but is higher than the 12.3 % and 13.9 % reported by RÖBBELEN and THIES (1980a) and BECKER et al. (1999), respectively. Conversely, the 7.2 % mean oleic acid recorded in this study is lower than what was reported earlier as 10.6 % by RÖBBELEN and THIES (1980a) and 9.8 % by BECKER et al. (1999). Like wise, the mean erucic acid content, which was about 33.4 %, obtained from this result is lower than earlier reports by RÖBBELEN and THIES (1980a), BECKER et al. (1999) and ALEMAYEHU and BECKER (2001). Except oleic acid, the other three fatty acids had population distribution in which less than 0.5 % of the plants belonging in the classes at both extreme low and high tail ends. In case of oleic acid, the proportion of the plants grouped towards the extreme high tail was lower than the extreme low tail. In general, the frequency distribution indicates the rareness of the desired variability for low linolenic and erucic acids and for high oleic acid. The variability of the four fatty acids observed in this study indicates that genotypes with a broad range of fatty acid composition could be selected out of the Ethiopian germplasm accessions. But the problem will be to combine all variations in one genotype with the desired level of each fatty acid regarded as optimum for human consumption.

The relationships of oleic with linolenic and erucic acids were negative. Since increased level of both linolenic and erucic acid is not nutritionally desirable, this relationship had a practical advantage towards selecting high oleic, but low linolenic and erucic acid containing genotypes. The positive association of linolenic and erucic acids is also advantageous as selection for reduced level of one of the fatty acids can be effected by selection for the reduced level of the other. Generally, except the positive, but low association of linolenic and erucic acids, the association between pair of fatty acids observed in this study agree with previous results of KUMAR and TSUNODA (1980) on 38 different species of *Brassicacae*; RÜCKER and RÖBBELEN (1995), RUDLOFF (1995) and MÖLLERS and SCHIER-

HOLT (2002) on *B. napus* and ALEMAYEHU and BECKER (2001) on *B. carinata*. Apart from the negative correlation between oleic and erucic acid that maintained in all species, RUDLOFF (1995) observed a very wide range of associations of both negative and positive values among the same pair of fatty acids in different *Brassica* species.

Evidence from QTL mapping in *B. napus* indicated that oil and protein in the seed shared large part of the same genetic basis with both pleiotropic and linked effects (ZHAO, 2002). To overcome the negative association between the two characters in *B. napus* RÖBBELEN and RAKOW (1979) (cited in RÖBBELEN and THIES, 1980a) proposed simultaneous improvement of both oil and protein content by selecting for their sum rather than for either component. The physiological reason behind this approach is to gain more oil and protein as a result of more effective partitioning of the available photosynthate into intrinsically more valuable products (RÖBBELEN and THIES, 1980a). Indexing protein and oil to their relative economic value, which in case of *B. carinata* the oil is more paying than the cake, could also help to solve the problem of the negative association between oil and protein.

Except the clusters formed by solitary plants, all the other clusters contained plants originated from more than one geographic area. Five of the 11 clusters were constituted by plants derived from accessions collected from all the eight geographic areas. Most of the plants derived from accessions of the same geographic origin dispersed in most of the clusters. For example plants derived from accessions collected from Welega (geographic area 7) spread across nine of the 11 clusters. This could ascertain the existence of genetic diversity within the same geographic area and also suggests that there were little or no barriers of gene flow between the different geographic regions. The characters studied could also have no or slight contribution towards geographic adaptation. Such an absence of direct correspondence between geographic and genetic diversities was also observed for other crops grown in Ethiopia: ABEBE and BJORNSTAD (1996) in barley, AYANA and BEKELE (1999) in sorghum and ALEMAYEHU and BECKER (2002) in *B. carinata*.

In conclusion, studying variability for quality traits in the segregating populations has unravelled the presence of rare but useful genes in *B. carinata* germplasm material, especially for glucosinolate content. High performance liquid chromatography analysis on seeds that showed low glucosinolate content confirmed the NIRS result but values from low glucosinolate containing genotypes were somewhat inflated (data not shown). Selection for low glucosinolate containing genotypes from the germplasm material is in progress. From this study, it had also been realized that no single plant was endowed with all the desired traits. Genetic recombination work should be made to combine the required quality traits into a single genotype and to pursue transgressive segregants for the traits in question. Genetic distance showed the presence of high level of genetic diversity with respect to the quality traits studied and their clustering pattern failed to show any direct correspondence between genetic diversity and geographic origin. Hence, the quality traits considered in this experiment have no or slight adaptive significance and quality improvement is not expected to have negative effect on adaptation of genotypes to specific environmental conditions.

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Address of the authors:

Adefris Teklewold, Holetta Research Centre, Ethiopian Agricultural Research Organization, Addis Ababa, Ethiopia P.O. Box 2003 (adechere@yahoo.co.uk); Present address, Georg-August University, Institute of Agronomy and Plant Breeding, Von-Siebold Straße 8, D-37075 Göttingen
Heiko C. Becker, Georg-August University, Institute of Agronomy and Plant Breeding, Von-Siebold Straße 8, D-37075 Göttingen, Germany (hbecker1@gwdg.de)