



**ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
DEPARTMENT OF BIOLOGY**

**DIVERSITY STUDY ON SEED QUALITY TRAITS OF ETHIOPIAN
MUSTARD (*BRASSICA CARINATA* A. BRAUN) AMONG SEED
SAMPLES SELECTED FROM OROMIYA REGIONAL STATE,
ETHIOPIA ASSESSED BY PROXIMATE ANALYSIS**

By
Abel Teshome Gari

*A Thesis Submitted to Graduate Studies Program, Addis Ababa University in
Partial Fulfillment of the Requirements for the Degree of Master of Science in
Biology (Applied Genetics)*

Addis Ababa
July, 2007

ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
DEPARTMENT OF BIOLOGY

**DIVERSITY ON SEED QUALITY TRAITS OF ETHIOPIAN
MUSTARD (*BRASSICA CARINATA* A. BRAUN) AMONG SEED
SAMPLES SELECTED FROM OROMIYA REGIONAL STATE,
ETHIOPIA ASSESSED BY PROXIMATE ANALYSIS**

By

Abel Teshome Gari

*A Thesis Submitted to Graduate Studies Program, Addis Ababa University in
Partial Fulfillment of the Requirements for the Degree of Master of Science in
Biology (Applied Genetics)*

Approved by Examining Board

.....
Internal Examiner

.....
External Examiner

Prof. Endashaw Bekele (Advisor)

Dr. Tesfaye Messele (Co-Advisor)

.....
Chairman

Acknowledgement

I would like to express my most sincere appreciation and gratitude to my advisors Prof. Endashaw Bekele and Dr. Tesfaye Messele for their keen interest, encouragements and unreserved guidance through out the course of the study.

I am also very much thankful to Dr. Adferis Teklewold, Dr. Kassahun Tesfaye to their advice and consultation during the thesis work. Furthermore, I would like to thank Ass. Prof Girmay Medhin for his professional support in the data management and analysis.

It is also grateful to thank the Institute of Biodiversity Conservation (IBC) for their goodwill to use their laboratory facilities. Appreciation is also further extended to Ethiopian Institute of Agricultural Research (EIAR), Holleta Agricultural Research Center, for providing the seed samples of *Brassica carinata*.

I would also like to give many thanks to my sisters Dr. Luwam Teshome and W/t Meron Teshome and dear friends Ato Serekebirhan Takele and w/t Kalkidan Biruk, Ato Yehenew Alemu and Ato Kaleab Ketema for their support and encouragement during the all times.

Finally, I would like to acknowledge the department of Biology of Addis Ababa University for accepting and training me as a postgraduate student.

Table of Contents

Acknowledgement.....	i
LIST OF TABLES.....	iv
LIST OF FIGURES.....	v
ABSTRACT.....	vi
1. Introduction.....	1
2. Literature Review	2
2.1 <i>Brassica carinata</i> : an overview.....	2
2.2 Taxonomy and Botany of Ethiopian mustard.....	3
2.2.1 The Genus <i>Brassica</i>	3
2.2.2 Phylogenetics	4
2.2.3 The Species Identity of <i>Brassica carinata</i>	6
2.2.4 Botanic Description and Reproductive Biology	7
2.3 Geographic Distribution and Habitat.....	8
2.4 Cultivation of <i>B. carinata</i> in Ethiopia	8
2.5 Economic Importance of <i>B. carinata</i>	11
2.6 Germplasm variability and its significance.....	12
2.6.1 Methods of Estimating Germplasm Variability.....	14
2.7 Seed Quality Traits of Ethiopian Mustard.....	14
2.8 Determination of Seed Quality Traits.....	15
3. Objective of the study.....	17
3.1 General objective	17
3.2 Specific objectives	17
4. Materials and Methods.....	18
4.1 Materials used	18
4.2 Sampling Technique	18
4.3 Nutritional Analysis.....	22
5. Data analysis.....	22
6. Results.....	23
6.1 Seed proximate composition of <i>B. carinata</i> and <i>B. napus</i>	24
6.2 Diversity in Seed Proximate Composition in Each Area.....	26
6.3 Correlation Analysis.....	33
6.4 Principal Component Analysis (PCA)	34

6.5	Hierarchical cluster analysis.....	39
7.	DISCUSSIONS.....	40
7.1	Variability in Composition of Seed Quality Traits of <i>B. carinata</i> populations from different Areas and Altitude Classes	40
7.2	Diversity in Seed Quality Traits of <i>B. carinata</i> within Each Area	41
7.3	Comparison between Released Cultivars and Populations of <i>B. carinata</i>	43
7.4	Correlation Analysis among the Six Proximate Compositions of <i>B. carinata</i>	44
7.5	Distribution of Samples of <i>Brassica</i> Oil seeds with Reference to the three Principal components	46
7.6	Cluster Analysis and its implication to the improvement of Ethiopian Mustard.....	47
8.	Conclusion and Implications for Breeding.....	48
9.	Recommendations.....	49
10.	References.....	51
	APPENDICES.....	60

LIST OF TABLES

Tables	Pages
Table 1 IBC accession number (acc.No.), regions, zone, woreda, altitude class (in meters), longitude and latitude of the samples included in the study and.....	19
Table 2 Cultivars of <i>Brassica</i> species used in the study	20
Table 3 Seed quality trait composition of populations of <i>B. carinata</i> and nationally released varieties of <i>B. carinata</i> and introduced varieties of <i>B. napus</i>	23
Table 4 Descriptive statistics of all seed samples in the study.....	24
Table 5 Correlation coefficient matrix between the six proximate compositions of the seeds used in the study.....	33
Table 6 Result of principal component analysis of <i>Brassica</i> oilseeds.....	34
Table 7 Component scores of coefficient matrix	34

LIST OF FIGURES

Figures	Pages
Figure 1 The relationship between diploid and naturally occurring amphidiploid species of <i>Brassica</i>	5
Figure 2 Yellow and Dark brown colored seeds of <i>B. carinata</i> populations used in the study.....	10
Figure 3 Site map of samples of <i>B. carinata</i> populations.....	21
Figure 4 The diversity of crude protein content in the seed Samples of <i>B. carinata</i> populations from three areas.....	27
Figure 5 The diversity of crude fat content in the seed samples of <i>B. carinata</i> populations from three areas.....	28
Figure 6 The diversity of fiber content in the seed samples of <i>B. carinata</i> populations from three areas.....	29
Figure 7 The diversity of mineral content in the seed samples of <i>B. carinata</i> populations from three areas.....	30
Figure 8 The diversity of moisture content in the seed samples of <i>B. carinata</i> populations from three areas.....	31
Figure 9 The diversity of carbohydrate content in the seed samples of <i>B. carinata</i> populations from three areas.....	32
Figure 10 The distribution of populations and cultivars of Ethiopian Mustard (<i>B. carinata</i>) and introduced cultivars of <i>B. napus</i> in relation to the first and second principal components.....	36
Figure 11 The distribution of populations and cultivars of Ethiopian Mustard (<i>B. carinata</i>) and introduced cultivars of <i>B. napus</i> in relation to the second and third principal components.....	37
Figure 12 The distribution of populations and cultivars of Ethiopian Mustard (<i>B. carinata</i>) and introduced cultivars of <i>B. napus</i> in relation to the first and third principal components.....	38
Figure 13 Dendrogram based on Euclidean distance for all six proximate composition of the seed samples of the populations and cultivars.....	39

ABSTRACT

This study was designed to generate information on the diversity of seed quality traits of samples of *B. carinata* populations. For this study, twenty one populations of *B. carinata* and three cultivars of *B. carinata* and additional three cultivars of *B. napus* were used. All the analyses were done according to the official methods of AOAC (Associations of Official analytical Chemists) international. The characterization of germplasm collections of seeds based on seed proximate composition has a special importance; it serves as a benchmark for identification of potentially improved parents for breeding works. In addition, the variability on the seed quality traits could be used to classify and assess the genetic relationship among populations of *B. carinata*. The study showed that the protein content ranges from 13.63 % to 23.88 % with a mean value of 18.67 %, crude fat content also showed a range from 30.36 % to the 52.19 % with total mean value of 45.80 %, the fibre content goes from 4.37 % to 13.5 % with mean value of 8.59 %, mineral content showed a range from 2.34 % to 4.01 % with a mean value of 3.29 %, moisture content ranged from 3.18 % to 5.09 % with the mean value of 4.23 % and finally, carbohydrate content showed a range from 21.36 % to 43.0 % with the mean value of 27.58 %. In the correlation analysis, the crude fat content of the samples showed negative correlation with that of the protein content and carbohydrate content. Moreover, the moisture content on dry weight bases showed a positive correlation with mineral content and protein contents and negative correlation with that of carbohydrate content. In contrast, carbohydrate content showed positive correlation with that of fibre content of the seeds. The principal component analysis generated three main components which explained around 80% of the total variation observed. In the cluster analysis, most of the cultivars used in the study fall in the same group, which indicates their consistency for the six seed quality traits analyzed in the study. Furthermore, the comparison of populations of *B. carinata* with that of the released cultivars in terms of seed quality traits revealed that there is still a room for enhancement of the cultivars. In general, the information generated from this study could be used to plan crosses and optimize the use of *B. carinata* here in Ethiopia and worldwide.

Keywords/Phrases: *B. carinata*, seed quality traits, protein, oil/fat, fibre, mineral, moisture, carbohydrate, proximate composition, seed quality traits.

1. Introduction

Ethiopia is one of the tropical countries found in east Africa. It has an area of 1.12 millions k.m², located between 3^o 24' and 14^o 53' North and 32^o 42' and 48^o 12' East. Its altitude ranges from 126 m b.s.l at Dankil depression to 4620 m a.s.l. at Ras Dashen. Furthermore, the country is endowed with a variety of agro ecological zones which in turn lead to rich fauna and flora. This makes the country an important center of diversity and endemism (Breitenbatch, 1963; Yalden, 1983).

The Ethiopian flora is estimated to contain between 6500 and 7000 species of higher plants of which 12 % are endemic (Tewoldebirhan, 1991). Thus, Ethiopia is one of the major centers of origin/diversity for leafy vegetables, root and tuber crops, spices, coffee and ornamental flowers. Consequently, plants like 'Enset' (*Ensete ventricosum*), 'Oromo dinich' (*Plectranthus edulis*) and 'Anchote' (*Coccinia abyssinica*) and (*Coffea arabica* L.) are believed to have been domesticated in Ethiopia, as a result of which has made significant contribution to the wide genetic diversity. Furthermore, vegetable crops such as shallot and garlic (*Allium* spp.), Ethiopian Mustard (*Brassica carinata*, A. Braun) etc. and some spices such as kororima (*Aframomum corrarima*), dill (*Anethum graveolens*), cumin (*Cuminum cyminum*) have either been indigenous or introduced long ago that their genetic base has been diversified in Ethiopia. (IBC, 2001).

In Ethiopia, there are no exhaustive records indicating the presence of diverse genetic resources in horticultural crops and others. However, the number of wild and semi-domesticated horticultural crops available in the country confirms the richness of the country in this respect. In addition to this, the wide cultural use of these crops, magnifies the fact that they had either originated in the country or been introduced to along time ago. Therefore, this further elaborates the fact that diversity of horticultural crops in Ethiopia, both between and within the species is immense (Messele, 2001).

Records of *B. carinata* are scarce here in Ethiopia or elsewhere, probably because it is rarely grown outside of Africa (Schippers, 2002). However, there is a tremendous amount of germplasm collections in the Institute of Biodiversity (IBC), but the technical information on the extent of its diversity is limited, especially concerning *B. carinata*. Therefore, the opportunity thereof to exploit the gene pool in line with improving its quality traits is virtually absent (Velasco, 2004). Thus, the purpose of this study is to investigate the diversity in populations of *B. carinata* based on their seed quality traits aimed at enhancing the utilization of the crop world wide.

2. Literature Review

2.1 *Brassica carinata*: an overview

In Ethiopia, the oilseed crops as a group constitute a considerable number and diversity of crop plants (Seegler, 1983). Oilseeds are those seeds that contain high percentage of oil. They do not belong to any particular group or family of flowering plants (Röbellen *et al.*, 1989). Seeds included in this category are groundnuts, soybean, Ethiopian mustard, palm kernel, cottonseed, olive, rapeseed, sesame seed, linseed, safflower, castor bean etc. Despite their importance, some of these seeds at present are not well known and thus may be grossly underutilized relative to their potential (Enujiugha and Ayodele-oni, 2003).

Among these underutilized oilseeds, Ethiopian mustard is one of them. It is grown as a vegetable and an oilseed crop in the highlands of the Ethiopia. It has also a potential as an oilseed crop in other countries like Canada (Rakow, 1995), Spain (Velasco *et al.*, 1995), India (Singh, 2003) and U.S.A (Cardone *et al.*, 2002). This is because the crop is better heat and drought tolerant than any other *Brassica* species. Therefore, it is well adapted to oilseed production under semi-arid conditions (BioMatnet, 2006).

In the plateaus of East Africa, Ethiopian mustard is one of the oldest crops known to exist (Simmonds, 1979). It is probably the only species of *Brassica* that can be considered as of

African origin, although there is a clear connection with Middle East and southern Europe types (Schippers, 2002). *B. carinata* is one of the major oil crops that has a significant place in the long-lived traditional agricultural practice of the country. This is because, the culture and cultivation of the crop is as old as the cultivation of cereals, which is believed to date back in the 4th and 5th millennia (Alemayehu, 2001).

Ethiopian Mustard (*B. carinata* A. Braun) is locally grown in most parts of the country situated above 1700 m.a.s.l. (Teklewold and Alemayehu, 1996). But, cultivation is mostly exercised by small farmers in more fertile and well drained fields, usually around homesteads. This may be due to its low oil and meal qualities. Nevertheless, at earlier stages of development the leaves and shoots of the crop are consumed as vegetable either by thinning or topping and seed can also be harvested from the plant for oil extraction and other traditional uses (Alemayehu, 2001; Tekelewold, 2005).

2.2 Taxonomy and Botany of Ethiopian mustard

2.2.1 The Genus *Brassica*

Ethiopian mustard (*B. carinata*) is a mustard crop from Ethiopia related to rapeseed (*B. napus*) (Panoutsou *et al.*, 1999). The flowering plant family Brassicaceae is known as the mustard family or cabbage family. The family consists of about 370 genera with nearly 3500 species (Edwards *et al.*, 2000). Among these species 75 of them are known to be found in Ethiopia (Seegler, 1983).

The pungent seeds of some species of this family lead the spice trade in the world today, (Hemingway, 1995). Apart from being used as a spice, mustards are widely used as leaf and stem vegetables and as a salad crop in Africa, Far East and Southeast Asia, and for green manuring or as a fodder crop mainly in Western Europe (Vaughan and Hemingway, 1959; Rosengarten, 1969). Nevertheless, the most important genus among the family members remains to be the genus *Brassica* (Brassicaceae, 2006).

The genus *Brassica* consists of 47 species. Members of this genus include many species economic importance that have been extensively altered and domesticated by humans. Their significance is magnified as a vegetable and oilseed crop; now being the World's third most important sources of vegetable oil (Downey, 1990; Kumar, 1995).

The ovules in *Brassica* species are bitegmic with each integument two-cell layers wide. The epidermis and parenchymatous layers develop from the two layers of the inner integument of the ovule (Setia and Richa, 1989). Although there is a large number of literature describing these species (Mulligan and Bailey, 1976; Ren and Beweley, 1998; Koul *et al.*, 2000), up to now little has been known about the seed coat patterns during whole seed development in *Brassica* species.

2.2.2 Phylogenetics

Brassica species were considered as species with fixed taxonomic entities and, alien gene transfer among different genomes were thought to be impossible. But attempts by Sinskaia in 1927, proved it wrong. He attempted a cross between *Brassica* species, but successful result was obtained by that of Karpechenko. Karpenchenko successfully synthesized *Raphano brassica*: the first inter-generic fertile hybrid of *Raphanus sativus* and *B. oleracea* in 1928 (Ahmed *et al.*, 2002). This practical result shed light on the possibility of genome manipulation of plants through wide hybridization program. Moreover, the artificial re-synthesis of *B. napus* (Yang *et al.*, 1998) during the second quarter of the 20th century resolved the debate of fixed taxonomic entities in *Brassica* species (Ahmed *et al.*, 2002).

Brassica is a polyploid complex having both elementary (diploid) and amphidiploid genomes (Yang *et al.*, 1998). Polyploidy is widely acknowledged as a major mechanism of adaptation which in turn leads to speciation in higher plants. About 70% of angiosperms are estimated to have experienced one or more episodes of polyploidization (Masterson, 1994; Jiang *et al.*, 1998).

Six of the most important *Brassica* species are closely interrelated. The elementary species include *B. rapa* (AA, 2n=20), *B. nigra* (BB, 2n=16) and *B. oleracea* (CC, 2n=18). The amphidiploid species include *B. carinata* (BBCC, 2n=34), *B. juncea* (AABB, 2n=36) and *B. napus* (AACC, 2n=38) (UN, 1935).

Studies on the origin of *B. carinata* indicate that it has evolved as a natural cross between *B. nigra* (BB, n=8) and *B. oleracea* (CC, n=9), followed by chromosome doubling in the highlands of Ethiopia and adjoining portion of East Africa and the Mediterranean coast where both the potential species were sympatric (Hemingway, 1995). This relation was elucidated with the artificial re-synthesis of the amphidiploids of *Brassica* (Gomez-Campo and Prakash, 1999).

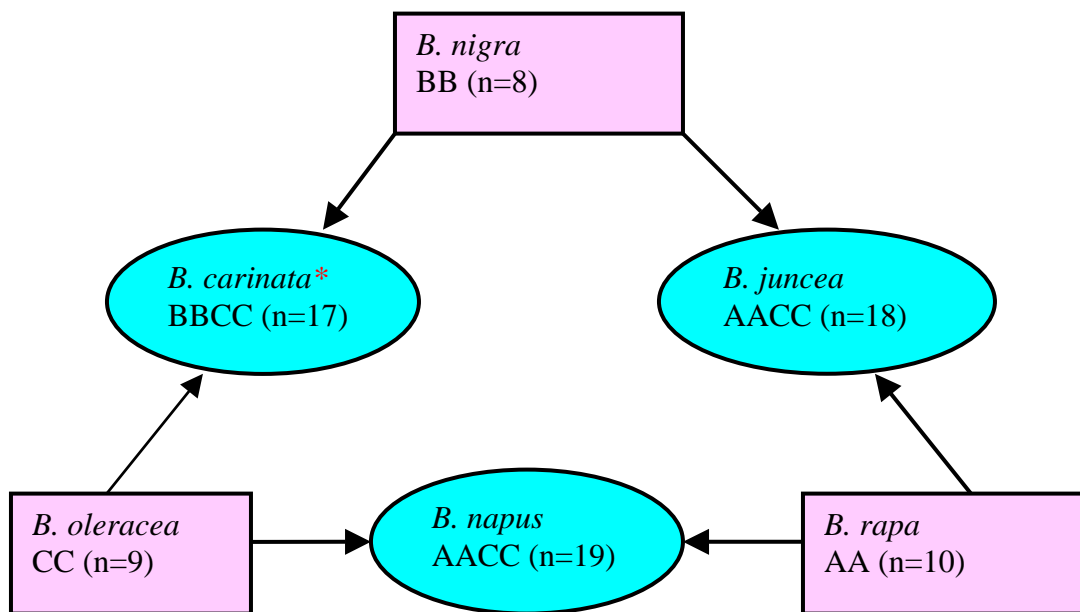


Fig.1 The relationship between diploid and naturally occurring amphidiploid species of *Brassica*.

In the figure (U's triangle), the three diploid species are represented at the tips of the triangle and their amphidiploids are found midway between the parental species. Letters with in parentheses denote haploid chromosome number of diploid and amphidiploid species.

Phylogenetically, *Brassica* is the nearest related genera to the recently sequenced *Arabidopsis thaliana* (Ahmed *et al.*, 2002). This species is becoming popular for understanding the *Brassica* phylogenetic relationship. The comparative maps of *Arabidopsis thaliana* and *B. rapa* showed that diploid *Brassica* species have hexaploid ancestors. Furthermore, variation in isozymes, chromosome numbers and the systematic relationships in Brassicaceae revealed aneuploidy and segmental polyploidy have played more significant role in the evolution of amphidiploid *Brassica* species. These elaborations encouraged breeders to integrate species diversity through wide hybridization for improved oilseed, vegetable fodders and forage *Brassica* crops (Yang *et al.*, 1998; IAR.1986).

2.2.3 The Species Identity of *Brassica carinata*

Current name: *Brassica carinata*

Authority: A. Braun

Scientific classification: Magnoliopsida: Dilleniidae: Capparales: Brassicaceae

B. carinata was given this name for the first time by A. Braun in 1841. The species is known to have various scientific synonyms like *Brassica intergrifolia* Var. *Carrinata* (West) Rupr (1860), *Melanosinapis abyssinica* Hort. ex Regel, *Sinapis abyssinica* A. Braun (1856) (Edwards *et al.*, 2000).

As the species originated and diversified in Ethiopia, it has different common names most referring to its origin. For example, Abyssinian Cabbage, Abyssinian Mustard, Ethiopian Kale, Ethiopian Mustard etc are some of them. However, locally it is famous by the name of and Gomenzere. Moreover, in different parts of the world it has different names like Figiri (East Africa), Loshuu (Tanzanian), Chambre dzagumana (Zimbabwe) Sukuma Wiki (Kiswahili), Tamu-texsel (Texas,U.S.A), Ethiopian rape seed (European trade mark name)(FAO, 2002; Schippers, 2002).

2.2.4 Botanic Description and Reproductive Biology

In Africa, the most widely consumed leafy vegetables are *B. carinata* and *B. juncea*. These two crops are frequently confused with each other due to their similarity in plant character and cultivation. Ethiopian mustard is an erect annual vegetable 0.9-1.2 m tall and 0.5m wide at flowering but, sometimes known to achieve a height up to 1.79 m (IENIEA, 2004; Mazzoncini, 1993). The leaves of the plant are usually thin, with many flowering shoots. The stem is reddish green, often profusely branched with lateral bud, and the leaves are alternate, non-heading with purplish veins and often light brown. Both the leaves and seeds are consumed. The leaves are cooked as a vegetable and the seeds are used for oil extraction. Those selected for there vegetables are often quite robust with thick stems and have large leaves and known to flower very late or none at all (Edwards *et al.*, 2000).

Regarding growth and development of the seeds, soils ranging from acid to alkaline and partial to full sun climate with moderate moisture are suitable for optimum growth of the seed. *B. carinata* is generally succulent in nature and grows mainly in gardens. Five days from sowing, the first leaf of the plant emerges. Then, new leaves emerge every three days. When the plant matures, it gives rise to flowers, which are yellow in color. Each flower is about 1.5 cm across, on short pedicles on an extended raceme. The flowers are regular with four free petals in one series and two sets of stamens. The fruit is a silique, usually less than 5 cm long, stout and broad with carinata angels, usually dehiscent. The seed is large and predominantly dark, often globular, 0.2 cm tick, filled with embryo (Edwards *et al.*, 2000; Andargie, 2006).

B. carinata has hermaphrodite flowers that are pollinated by bees, and it is self pollinating. This feature makes it similar with *B. juncea* and *B. napus* of the genus (Nei, 1978; Pandey *et al.*, 1999). Furthermore, intergeneric crosses are also possible between various *Brassica* species and other crucifers (e.g. *Raphano brassica*) this is due to the fact that six of the most important *Brassica* species are closely interrelated. Thus, the crosses can be used for

human benefit, to transfer better agronomic traits from one genus of *Brassica* to another (Pandey *et al.*, 1999).

2.3 Geographic Distribution and Habitat

B. carinata is found exclusively in Ethiopia but, recently it has been cultivated in different parts (corners) of the world. Nowadays, it is grown in Central Africa (Zambia), West Africa (Sera Leon and Guinea) and Asia (India, China, Bangladeshi and Indonesia) as a vegetable crop along with other members of the genus. This crop is also extensively cultivated in Eastern Europe and U.S.A. as animal fodder (FAO, 2002).

Ecological heterogeneity in terms of altitude, rainfall, soil, etc has contributed to the emergence of diverse vegetations and indigenous food plants in Ethiopia (Asfaw, 1992). *B. carinata* is one of such indigenous food plants grown (cultivated) extensively in the highlands. But, there are also weedy forms of *B. carinata* growing in the highlands of the country which are gathered to be eaten as leafy vegetables (PGR, 1995; Schippers, 2002).

Ethiopian mustard is grown in tropical lowlands, but more common in mountainous regions that are above 700 m a.s.l. The Central and Northern highlands of the country especially, altitudes above 1200 m a.s.l. are famous for cultivation of this crop. In cultivated areas, higher yield is obtained between 1500 and 2600 m a.s.l. A well distributed rainfall between 1000-1500mm is adequate for the cultivation (FAO 2002; Schippers, 2002).

2.4 Cultivation of *B. carinata* in Ethiopia

Oilseeds are important agricultural commodities widely grown in Ethiopia. Major oilseeds include Gomenzere, sesame seed, groundnuts, Soybeans (partly used for oil extraction), rapeseed, Noug, linseed, sunflower, cottonseed and others. According to Gomez-Campo and Parkash 1999), cultivation of Ethiopian mustard in the plateaus of the country goes to

antiquity. It has been grown as vegetable and an oil crop in Ethiopia. But, production is consumed locally with no promotion to international trade (Hemingay, 1995).

Experiments indicate that the crop performs well in irrigation than in natural rain condition. This is due to less incidence of pest attack at irrigation fields (Alemayehu, 2001). The species is drought resistance but also perform better in fertile, sandy, loam-soils rich in humus and responds well to soil fertilization and manuring (FAO, 2002). *B. carinata* is normally cultivated during the rainy season in the tropics and sub-tropics. The African types are well adapted to high temperature and produce large quantity of seeds (FAO, 2002).

The survey carried out on the productivity of *B. carinata* revealed that the highest amount of the crop produced is about 1.13 tones hectare⁻¹. But it is grown only on 17,256 hectares all over Ethiopia. This statistics makes *B. carinata* the third smallest area cropped by the six species of oilseeds grown in Ethiopia (CSA, 2003). However, between the year 1982 and 2003, the production trend analysis indicates that there is a tremendous increase in area and production of the crop. The increase was 575 % and 1044 % respectively. Recently, farmers have shown little interest in crop, but this attraction could be further enhanced by developing varieties with better agronomic qualities (Teklewold, 2005).

Ethiopian Mustard is known for its agronomic qualities which are rare or absent in other oilseed *Brassica* species. These include relatively large seed size (Getinet *et al.*, 1996), high heat and drought tolerance, good resistance to black disease and reduced amount of seed shattering problem (Gugel *et al.*, 1990). It is also resistance to insect pests like aphids and flea beetles (Bayeh and Gebremedhin, 1992), and some accessions have high levels of resistance to alternaria black spot (Yitbarek, 1992). Moreover, Ethiopian mustard is a promising oilseed crop for semi-arid areas where it has better agronomic performance than its close relative's rapeseed (*B. napus* L.) (Deharo *et al.*, 1998).

Thus, comparatively Ethiopian mustard possesses a number of agronomic advantages over other *Brassica* species (Alemayehu and Becker, 2002). However, like other *Brassica* species, naturally occurring Ethiopian mustard forms are characterized by the presence of a high concentration of erucic acid in their oil (Velasco *et al.*, 1998). This feature makes it harmful for human consumption if it is taken in large doses. Therefore, efforts have been made to develop low or intermediate erucic acid genotypes of *B. carinata* using different strategies (interspecific crossing, mutagenesis etc) (BioMatnet, 2006).

Furthermore, recently the cultivars of Ethiopian mustard gained the interest of researchers in Canada and Spain because of their robust agronomic qualities. For example, yellow seeded *B. carinata* is important for introgression of genes into *B. napus*, where there is no yellow seed character (Chen *et al.*, 1989b; Alemayehu and Becker, 2002; Kimber, 1995). This yellow seeded *B. carinata* is known to have a thinner seed-coat. Therefore, it promotes water absorption and successful seed germination in arid conditions. In addition to that, it is associated with higher oil protein and lower crude fibre contents. This seed color is believed to be a maternal trait because it arises from the outer integument of the ovule and multiple loci control the biosynthesis of the seed pigment (Chen *et al.*, 1989a). Hence, *B. carinata* can serve as an important source of genes for a breeding program aimed at improving the performance of other oil seeds in general (Tsigie *et al.*, 2005a).



Fig. 2 Yellow and dark brown colored seeds of *B. carinata* populations used in the study.

2.5 Economic Importance of *B. carinata*

B. carinata is an oil crop with plenty of use values since ancient times in Ethiopia. Traditional applications of the crop occupy a pride place in country (Alemayehu, 2001). Since the time of our forefathers, it has saved the lives of many Ethiopians. This is because in localities where there is a shortage of grain in the annual cycle of production, when the families have little or no stored supply of food before the next harvest time, the shoots and leaves of the crop are used to sustain on. This is due to the fact that *B. carinata* needs less time of harvest compared to other vegetable crops locally grown. Moreover, its adaptation in semi-arid environment makes it an ideal candidate in a country like Ethiopia where drought is a common feature at intervals. Due to this feature of the crop being more consumed or needed at the time of trouble, some local people advocate that eating of this vegetable crop as a sign of shortage of food or famine, despite of the fact that it has important nutritional qualities (Asfaw, 1992).

In general, the nutritional content of the meal (food) consumed in Ethiopia is low in vegetable oil or animal fat. The expected amount of fat/oil is 20-25 % of the total caloric intake (FAO, 1988). Among other reasons, the low income of most members of the society makes them unable include meat and meat products in their daily meal is the main cause. Moreover, the absence of habit of eating vegetables as part of the meal is another. Thus, to upgrade the malnutrition problem, especially that of oil/fat and protein contents, plant seeds can be an ultimate choice in poor countries like Ethiopia. Seeds have nutritive values, which makes them necessary in diets. They are good sources of edible oils and fats. Therefore, Ethiopian Mustard can be an alternate choice by improving the oil and protein contents of an already adapted high yield giving oilseed varieties (Alemayehu, 2001). Furthermore, adding Ethiopian mustard to everyday meal as a vegetable is advantageous. This is because; it has special nutritional components like vitamins, minerals, trace elements, dietary fibre and protein. It also gives zest and flavor of diets (Asfaw, 1992; Tsige *et al.*, 2005b).

In addition to the general use of the crop as apart of meal, it also serves the purpose of being used for oil extraction along with other oilseed crops (Alemayehu, 2001). Furthermore, in native Ethiopia the ground seeds are used to lubricate Enjera and bread baking traditional clay-pan. Moreover, the powder of the seed is used to prepare beverages and cure certain illness like stomach upsets. In some areas, the crop is used as a green manure. The crop has also benefits in traditional farming system for crop rotation. It serves as the break crop for the cultivation of cereals with comparative ecological amplitude (Zelege and Mariam, 1991).

Despite its diversity, deep-rooted traditional uses and better adaptive features, however, until recently it has never been known as full-fledged field crop. Its cultivation so limited, and grown as garden crop around homestead or sparsely mixed with thick crop stands of maize, sorghum, teff and finger millet (Velasco *et al.*, 2004).

Even though, it has been forgotten in the line of research, a basic experimental study on non-traditional use of the crop has given positive results. For example, after transesterification the oil exhibit physical and chemical properties suited for bio-diesel (Cardone *et al.*, 2002). In addition, it can be used as feedstock for oleo chemicals (due to its high erucic acid and linoleic acids) and bio-fumigant industries (due to its high glucosinolate) (Teklewold, 2005). In general, *B. carinata* have proven to be one of the few commercially important oilseed plants to responds to biotechnologies quickly (Pandy *et al.*, 1999).

2.6 Germplasm variability and its significance

Genetic diversity of cultivated plants and domestic animals is due to the non-stop interaction of mankind and the environment. The evolutionary forces (mutation, selection, and random genetic drift) are the main natural causes of genetic diversity. Furthermore, the struggle for survival by our ancestor's lead to the domestication, selection and finally production of crop species those are unique to the country. In general, the cause of high

diversity among germplasm accessions of domesticated species is thus both environmental and man-imposed (Bekele, 1985).

In domesticated crop species, populations of different geographical origin show that there is a selection pressure in a particular environment and less selection pressure in other environments. This is especially true in countries like Ethiopia where there are many nations and nationalities which in turn lead to high cultural diversity. This cultural versatility results selection of different crops at different localities as a source of food and for other applications. Thus, this variation in selection pressure finally, causes inherent variation among different populations of the same species (Hawtin *et al.*, 1997).

In Ethiopia, the genetic resources of traditional vegetables are to a large extent left to traditional process, although they are presumed to have high potential (Zelege and Mariam, 1991; Dessalegn *et al.*, 1994; Asfaw, 1992). *B. carinata* is one of those traditional vegetables left out of the main line of research and studies.

B. carinata which is among the underutilized crop species of antique heritage believed to have originated in the plateaus of Ethiopia (Chen *et al.*, 1989a). Recent studies on this species indicated that it has agronomically important genes of rare occurrence. *B. carinata* is known to be unique for its yellow seeded types, which is absent in other *Brassica* species. Liu (2003) reported the occurrence of a dominant Yellow gene (Rp) in *B. carinata* that inhibited or repressed the expression of usual brown seed coat color.

But, to take advantage of these important traits, the diversity and eco-geographic pattern of inheritance should be studied. In addition to that, the regions with high diversity as well as those endowed with useful agronomic traits needs to be identified for core collection of the plant. Furthermore, the pattern of seed quality variability among the population of *B. carinata* need to further assessed so as to make use of the genetic resources in the country (Ahmed *et al.*, 2002).

2.6.1 Methods of Estimating Germplasm Variability

Characterization of genetic diversity can be carried out at all levels of individuals i.e. populations, species, genera, families and higher taxonomic levels (Nei, 1978). This quantification of genetic diversity and studying the eco-geographic patterns of the variability are of prime importance in plant breeding, germplasm conservation and for basic studies in crop evolution. This is due to the fact that the information gained enables breeders to choose parents of potential use in the breeding programs and for gene banks to enhance their efficiencies (Bhatt 1970; Jain and Singh 1972; Jain *et al.*, 1975; Arunachalam, 1981).

Biometric analyses of quantitative and qualitative traits are used for evaluating diversity of a plant. Among these, seed quality trait analysis by itself or in conjunction with other markers like phenotypic evaluation can be used for characterization of a plant of interest (Maria *et al.*, 2004).

2.7 Seed Quality Traits of Ethiopian Mustard

Oil extracted from plant seeds are of importance in nutrition, industries, and in pharmaceutical sectors (Dagne and Jonsson, 1997). The nutritional importance of a vegetable oil is dependant on it's a vegetable composition and other oilseed quality traits (McVetty and Scarth, 2002). The oil of *B. carinata*, like those of other oilseed *Brassica*, contains 35 – 44 % erucic acid (22:1), but as opposed to most other vegetable oils, it contains fewer amounts of the fatty acids with 16-c and 18-c atoms (Westphal and Marquard 1980; Röbellen and Thies 1980a; Röbellen, 1981; Downey 1990; Becker *et al.*, 1999). This feature of the oil i.e. high content of erucic acid and low content of fatty acid composition makes it despised as part of a meal for humans and animals fodder.

In 1940's and 50's experiments carried out on rats upon feeding on meals containing high amount of erucic acid implied it has adverse effects. These effects include poor

digestibility, weight loss, myocardial lipidosis, even death. Thus, using *B. carinata* as a source of vegetable oil, with its nutrient composition at present might result adverse health problems (Sauer and kramer, 1983).

Therefore, by applying quality breeding it is possible to upgrade the nutritional content of Ethiopian mustard and take advantage of these and other important traits of the crop. In order to achieve this goal, there should be continues search for new genetic resources of useful oil content as well as characterization. As a result, currently, the seed quality traits of many plant species are being chemically analyzed as a new oil crop (Hirsinge, 1989). Furthermore, analysis of the patterns of seed quality traits among groups of the plant species also proved to be useful in chemotaxonomy and phylogenetic studies (Graham *et al.*, 1981; Velasco and Goffman, 2000).

In Canada and Western European countries, rapeseed (*B. napus*) has become major source of vegetable oil and oilseed protein by improving its seed quality traits. This was done by altering its fatty acid profile such that linoleic and erucic acids from the oil and glucosinolates from the meal get reduced to suit human and animal nutritional requirements (Röbellen and Thies, 1980b; Becker *et al.*, 1999). Similar nutritional improvement has also been applied to *B. juncea*. Now, current efforts are undertaken in Ethiopia and elsewhere, to improve the nutritional content in the oil of *B. carinata* and reducing glucosinolates content in the meal (Rakow, 1995; Getinet *et al.*, 1996; Velasco *et al.*, 1998; Alemayehu and Becker, 2002).

2.8 Determination of Seed Quality Traits

Since most plant breeding methods both traditional and modern have the potential to alter the nutritional value of plants, they may lead to unexpected or unintended changes in chemical composition of the plant (Agbios, 2001). Therefore, assessing the chemical composition of the plant before and after manipulation is vital from many perspectives.

These include nutrition and health, toxicology and safety and stability to morphological, chemical or physical changes.

In many nutrition laboratories, most of the routine work of seed quality determination comprises methods of proximate analysis. Proximates are usually considered to be the nutrients which are present in amounts greater than 1 %. These include moisture, fat, protein, fibre, mineral ash and carbohydrate contents (Ferris *et al.*, 1995). The carbohydrate content is usually determined by the difference from the original sample minus the moisture, protein, crude fat, and mineral content with their contents calculated at the same moisture level (Raghuramulu *et al.*, 1983). Furthermore, the nutritional study also deals with that of additives and contaminants. Nevertheless, the main compositional components of interest are the proximate compositions. (Krik and sawyer, 1991).

The Proximate analysis is widely accepted as a basis for nutritional evaluation of seeds. In addition to that, it helps to group seeds according to their seed chemical composition similarity and relationship. The analysis also plays an important role in the assessment of seed quality and safety, both in industry and for enforcement of authorities at national and international levels (Krik and Sawyer, 1991).

Studies concerning the genetic diversity of *B. carinata* populations are very limited. However, these limited studies on populations collected from different parts of Ethiopia showed the presence of wide variation for morphological and agronomic traits (Abebe *et al.*, 1992). Furthermore, multidisciplinary study on genotypes of Ethiopian mustard by Alemayehu and Becker (2002), revealed the presence of large genetic diversity for preferred agronomic traits and the presence of considerable amount of variations in oil, and protein, fatty acids, and glucosinolates contents in germplasm accessions (Tsigie *et al.*, 2005b).

3. Objective of the study

3.1 General objective

To generate information on the diversity of Ethiopian Mustard (*B. carinata* A. Bruan) population based on their seed quality traits.

3.2 Specific objectives

1. To determine level of variability for six seed quality traits among the populations of *B. carinata* by origin and altitude classes.
2. To see the correlation between the six seed quality traits of *B. carinata*.
3. To classify *B. carinata* populations and asses their relationship among them based on seed quality traits.
4. To compare the seed quality traits of the nationally released varieties *B. carinata* with that of the landrace populations of *B. carinata* and with introduced cultivars of *B. napus* in order to select parents of desired proximate composition.

4. Materials and Methods

4.1 Materials used

A total of twenty seven seed samples of *Brassica* oilseeds were used for this study. Among these twenty one were populations of *B. carinata* collected from different areas in Oromia administrative state. Furthermore, three released varieties of *B. carinata* and additional three introduced varieties of *B. napus* were included for comparison. The materials for this study were obtained from Ethiopian Institute of Agricultural Research Organization Holleta Agricultural Research Center. The seed samples were obtained from the populations that were cultivated at Holleta Research center in the 1999/2000 main cropping season. Each sample is from different plants of each accession, which were analyzed for seed quality trait. Furthermore, the germplasm collections represent different altitude classes of the localities under consideration. A detailed description of the materials used in this study is shown in Table 1.

4.2 Sampling Technique

In this study, twenty one seed samples from three areas of Oromia were used. During the selection of the twenty one populations from different localities in Oromia administrative region were considered. Furthermore, within the selected localities different altitudinal classes were considered to have a representative samples of the populations found there. The three localities were selected based on the fact that most collections of the species in IBC were from these localities. Based on this benchmark, the preferred areas for the study were Wellega (Western Oromia), Shewa (Central Oromia) and Harar and Bale (Eastern Oromia). Each area was then divided in to four altitudinal classes. Finally from each locality for the first three altitudinal classes two samples were taken and one sample is taken from the last altitudinal class. In general, seven samples are taken from each area chosen for the study.

Table 1 IBC accession number (acc.No.), regions, zone, woreda, altitude class (in meters) longitude and latitude of the samples included in the study.

S/N	Acc. No	Zone	Woreda	Latitude	Longitude	Altitude	Altitude Class
1	202945	E. Shewa	Adama			1600	1
2	208585	E. Shewa	Boset	08-35-N	39-12-E	1600	1
3	203223	W. Shewa	Ambo			2160	2
4	203225	W. Shewa	Addu gerga			2200	2
5	203230	W. Shewa	Alem gena			2280	3
6	212224	N. Shewa	Wara Jarso	10-00-N	38-15-E	2520	3
7	20130	N. Shewa	Gerar Jarso	08-49-N	38-45-E	2750	4
8	207919	W. Wellega	Hawa Welele	08-44-N	35-00-E	1550	1
9	207918	W. Wellega	Lalo Agabi	09-10-N	35-41-E	1800	1
10	215790	E. Wellega	Sayo	8-3-N	34-46-E	1950	2
11	21094	W. Wellega	Gida Kerimu	09-55-N	37-20-E	2090	2
12	21217	E. Wellega	Jima Horo	09-12-N	37-12-E	2320	3
13	200405	E. Wellega	Jimma Arjo			2330	3
14	208961	E. Wellega	Jimma Horo	09-20-N	37-20-E	2700	4
15	208594	E. Harar	Goro Gutu	09-06-N	40-21-E	1750	1
16	208590	W. Harar	Chiro	08-53-N	40-33-E	1770	1
17	208599	E. Harar	Kombolcha	09-03-N	42-00-E	2100	2
18	208611	E. Harar	Kersa	09-10-N	41-32-E	2180	2
19	208592	Harar	Tulo	09-18-N	41-07-E	2300	3
20	21070	Bale	Sinana dinsho	07-13-N	39-51-E	2630	3
21	21076	Bale	Goba	07-02-N	39-56-E	2750	4

Key:

Altitude class 1: 1450 – 1850
Altitude class 2: 1851 – 2250
Altitude class 3: 2251 – 2650
Altitude class 4: 2651 and above

Table 2 Cultivars of *Brassica* species used in the study.

	Cultivars	Species	Origin
22	Yellow-Dodolla	<i>B. carinata</i>	Ethiopia
23	Holleta- 1	>>	>>
24	S-67	>>	>>
25	Pura	<i>B. napus</i>	Europe
26	Tower s-3	>>	>>
27	Tower	>>	>>

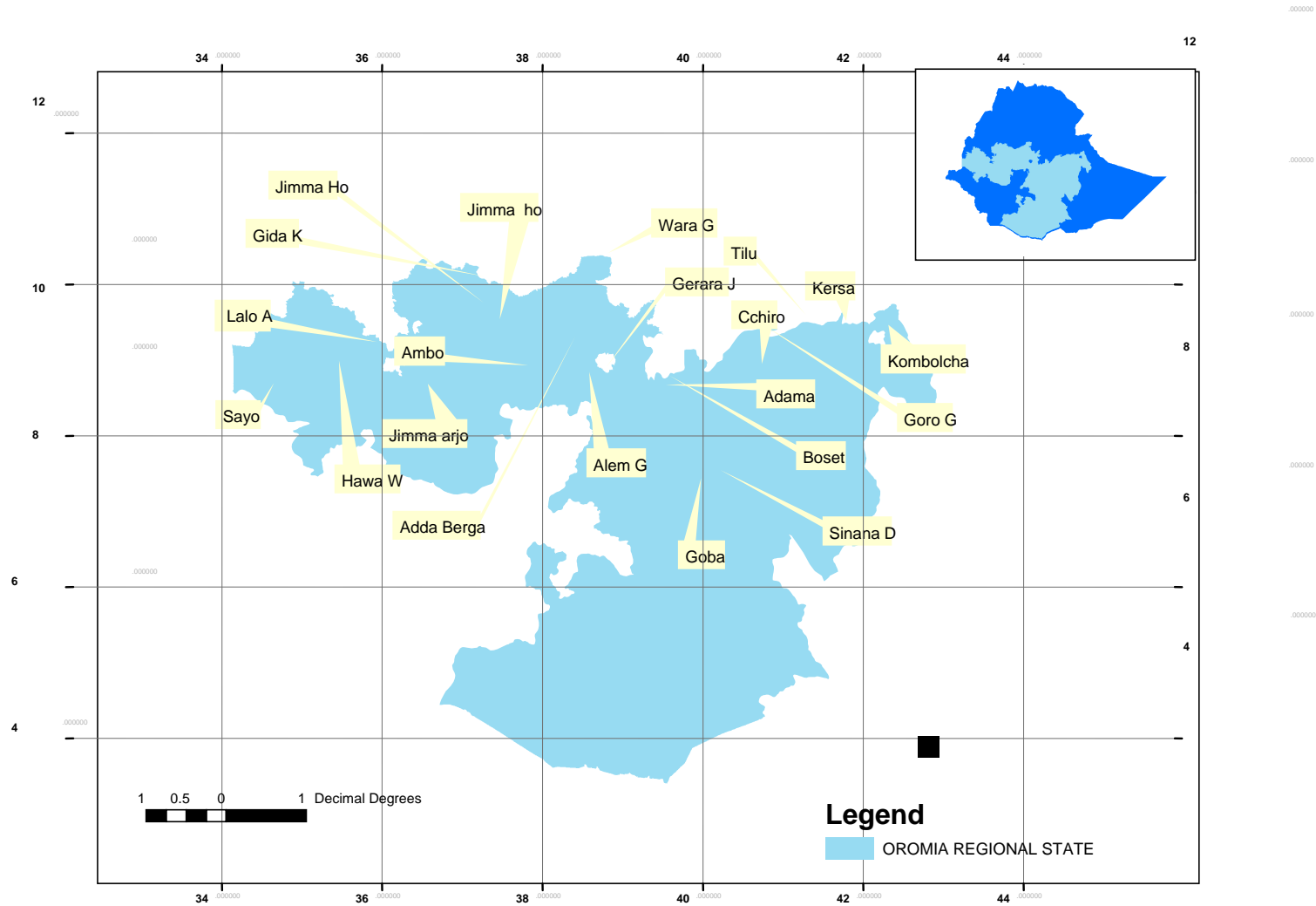


Figure 3 Site maps of samples of *B. carinata* populations.

4.3 Nutritional Analysis

Approximately 30 g of seed from each population were ground in ultra centrifugal mill ZM 1000 with 0.5 sieves and analyzed in triplicate. Data was recorded for six seed quality traits, namely: crude protein, crude fat/oil, fibre, mineral ash, moisture and carbohydrate contents.

All analyses were done according to official methods of analysis of AOAC (Associations of Official analytical Chemists) international (AOAC, 1996). The seed's crude protein content was determined by taking 0.25 g of sample and measuring the nitrogen released using Kjeldah method. Then percent of N obtained was multiplied by a factor of 5.53 (Tkachuk, 1969). Crude fat/oil content was determined by extracting 3 g of each sample with Petroleum Benzene on Soxtec extraction unit. Fibre content in seed samples is usually measured as crude fibre, determined by the loss in mass upon ignition of the dried residue remaining after 1 g of the sample being digested with dilute sulfuric acid (0.128M) and potassium Hydroxide (0.223M). Mineral content was determined by taking 3 g samples and incinerating it in a muffle furnace at 55⁰ C over night. Moisture content was determined by taking 2 g sample and putting it in an oven at 103⁰ C for seventeen hours and then, taken out of the oven and cooled in disketer and is weighed after while. Finally, the carbohydrate content of the samples was calculated by difference (carbohydrate = 100 - (Protein + Fat + Mineral + Moisture). All proximate compositions were analyzed and calculated on dry weight basis (dwb).

5. Data analysis

Statistical analysis was carried out using SPSS version 13.0 soft ware. A significance level of 0.05 was used for all tests.

6. Results

Seed quality traits analysis of populations and released cultivars of *B. carinata* and introduced varieties of *B. napus* was carried out in this study. The analysis revealed the presence of significant variation in most of the six proximate compositions of the samples, but to a different extent. The result of the analysis is summarized in the table below.

Table 3 Seed quality traits composition of populations of *B. carinata* and nationally released varieties of *B. carinata* and introduced varieties of *B. napus*.

S/N	Accession No.	Region of origin	Proximate compositions of the seed samples					
			% crude Protein	% crude Fat	% crude Fibre	% Mineral ash	% Moisture	% Carbohydrate
1	202945	Shewa	17.1769	44.5008	8.3371	3.6572	4.5002	30.1648
2	208585	>>	20.2221	31.6196	10.3484	2.8308	4.0121	41.3153
3	203223	>>	19.2621	46.5382	6.1604	3.3083	4.5970	26.2944
4	203225	>>	18.3124	47.7448	8.9044	3.4842	3.9306	26.5279
5	203230	>>	17.2801	47.4898	12.56	3.2334	4.0740	27.9227
6	212224	>>	19.6337	49.8556	6.9826	2.7057	4.0086	23.7964
7	20130	>>	17.4040	49.4050	6.0501	3.5847	3.8986	25.7076
8	207919	Wellega	16.9808	47.0014	10.1943	2.8296	4.0871	29.1011
9	207918	>>	17.1150	47.0052	7.6923	3.4298	4.1895	28.2605
10	215790	>>	18.7769	46.9877	8.6704	2.5522	4.1047	27.5785
11	21094	>>	22.8647	41.3474	8.4006	3.3166	4.5022	27.9691
12	21217	>>	17.7343	45.5543	6.5533	3.7961	4.1124	28.8028
13	200405	>>	14.5550	50.3146	6.0867	3.2107	3.3975	28.5223
14	208961	>>	18.2195	47.7262	9.2097	3.2441	3.8039	27.0064
15	208594	Harar	16.5885	49.1325	8.2586	3.4095	4.3992	26.4703
16	208590	>>	19.6131	46.3451	9.4232	2.9151	4.4585	26.6683
17	208599	>>	19.3447	47.3924	10.1227	2.8041	4.1271	26.3317
18	208611	>>	22.5757	44.3509	8.8518	2.6215	4.4076	26.0442
19	208592	>>	19.8092	45.1952	4.9108	2.8138	4.1760	28.0059
20	21070	Bale	20.9034	43.4012	7.9420	3.7100	4.5420	27.4434
21	21076	>>	20.7692	40.8224	13.3609	3.8558	4.0076	30.5450

Table 3 continued

22	Yellow Dod.	17.6724	49.5731	7.6353	3.3004	4.0342	25.4199
23	Holleta-1	18.5705	45.3016	8.0302	3.9742	4.4845	27.6692
24	S - 67	18.0337	47.0291	9.1575	3.9591	4.7323	26.2458
25	Pura	20.2428	48.0021	8.7051	3.5014	4.2109	24.0429
26	Tower-s-3	15.4732	50.0655	9.3458	3.3759	4.2491	26.8363
27	Tower	18.7872	48.5580	10.1129	3.5631	5.0566	24.0350

6.1 Seed proximate composition of *B. carinata* and *B. napus*

Table 4 Descriptive Statistics of all seed samples in the study.

Proximate composition	N	Range	Minimum	Maximum	Mean	Std.error	Variance
Protein	27	10.25	14.555	22.8647	18.6637	0.2236	4.050
Fat	27	21.82	31.6196	50.3146	45.8027	0.5698	26.295
Fibre	27	9.13	4.9108	13.3609	8.5927	0.2196	3.908
Mineral	27	1.67	205522	309742	3.2958	0.0458	0.170
Moisture	27	1.91	3.3975	4.5970	4.2261	0.0406	0.134
Carbohydrate	27	21.65	23.7964	41.3153	27.5825	0.3641	10.738

N is the number of samples used in the analysis.

This study showed that the protein content ranged from 13.63 % to 23.88 % with a mean of 18.81 %. In the case of fat content, the highest value is 52.19% and the least crude fat content is 30.36 % with total mean value of 45.80 %. In both cases of the proximate composition, there exists significant variation in samples analyzed. Furthermore, the fibre content of the crop among the samples under consideration goes from 4.37 % to 13.5 % with mean value of 8.59 %. Other seed quality traits also showed significant variation among the seed samples, mineral content ranged from 2.34 % to 4.01 % with a mean value of 3.29 %. In the case of mineral content the range between the samples was narrower than

but still there is a significant variation among seeds. The moisture content also revealed a range from 3.18 % to 5.09 % and with the mean value of 4.23 %. Yet again the range is narrow but significant variation exists between the samples considering their moisture content. Finally, the carbohydrate content showed significant variation among the samples under study. The carbohydrate content ranged from 21.36 % to 43.0 % and with the mean value of 27.58 %. In general, similar phenomenon is observed in the proximate contents i.e. significant variation exists between the seed samples used in the study (Appendix, 3).

F-test was carried out to see if variation exists in seed quality trait among the seeds of *B. carinata* collected from different localities under consideration. In the case of fat, mineral, fibre and carbohydrate contents the between group analysis showed no significant variation among the areas considered. Concerning the protein and moisture contents the F-test showed that there is a significant variation between the areas at $P \leq 0.05$ level. Thus, to find out where the variation lies Post Hoc test was carried out further. The analysis confirmed that there is significant variation between Shewa and eastern Oromia and that of Wellega seed samples. In each case of protein content of samples from Eastern Oromia were higher than that of the samples from the two areas under consideration. Yet again, in the case of moisture content seed samples from Eastern origin became higher than the samples from the other localities. But, significant variation was obtained between the seed samples of Shewa and Eastern Oromia only (Appendix 2).

Furthermore, F-test was conducted to investigate the presence of seed proximate content variation among the altitude classes from which the seeds samples were originally collected. In this analysis, only the first three altitudinal classes were chosen for comparison because of their equal sample sizes (Table1).

Therefore, the test revealed significant variation for some of the quality traits. These are Protein, fibre and carbohydrate contents (Appendix 5). However, for protein content of the samples, the populations from the second altitude class were relatively higher than the other two classes. Moreover, in the case of carbohydrate content, the populations from the

first class have got higher amounts of the proximate composition. In general, in all altitudinal classes compared and no class has been observed with an exaggerated value of most of the seed quality traits. (Appendix 4).

6.2 Diversity in Seed Proximate Composition in Each Area

For each area considered in the study, the diversity observed for six quality traits was different. The box plots revealed that the variation among the area concerning the diversity of each seed quality traits. The results are summarized in the figures below (Fig. 4 - 9).

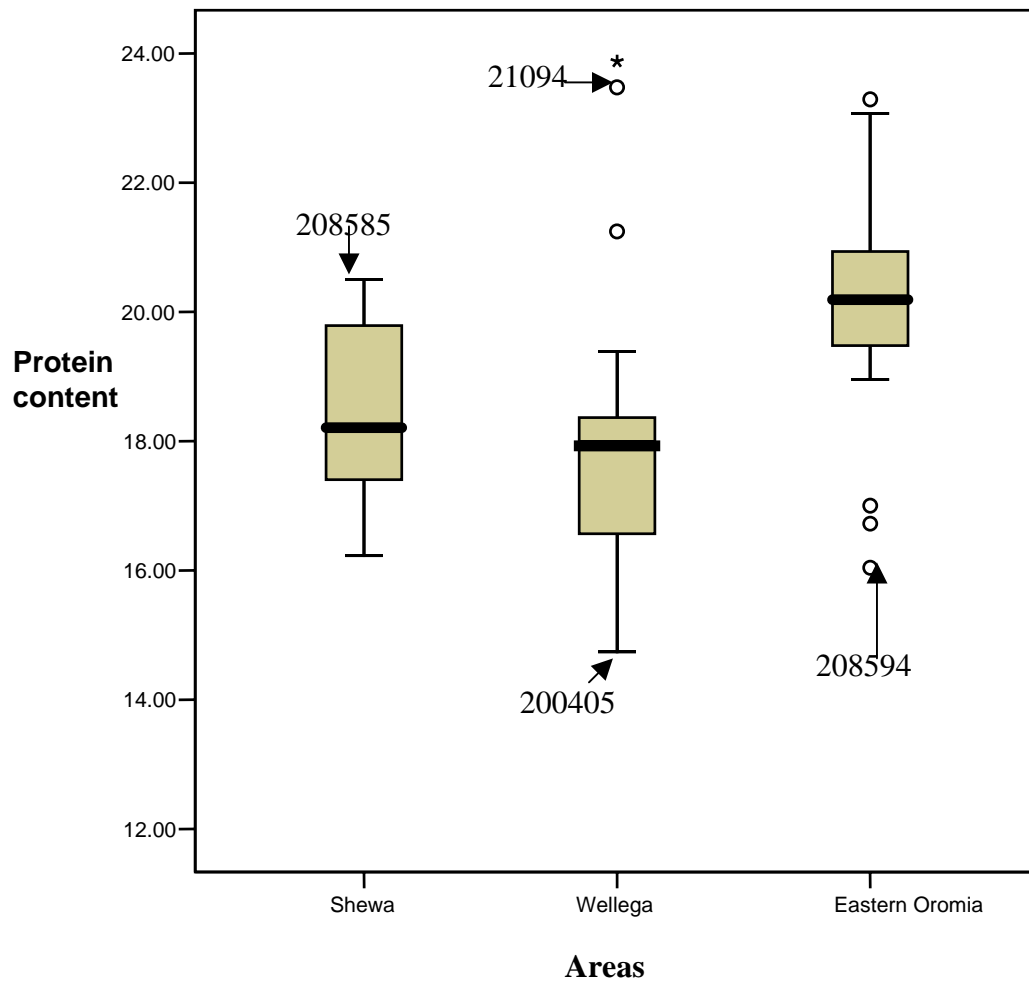


Fig. 4 The diversity of crude protein content in the seed samples of *B. carinata* populations from three areas.

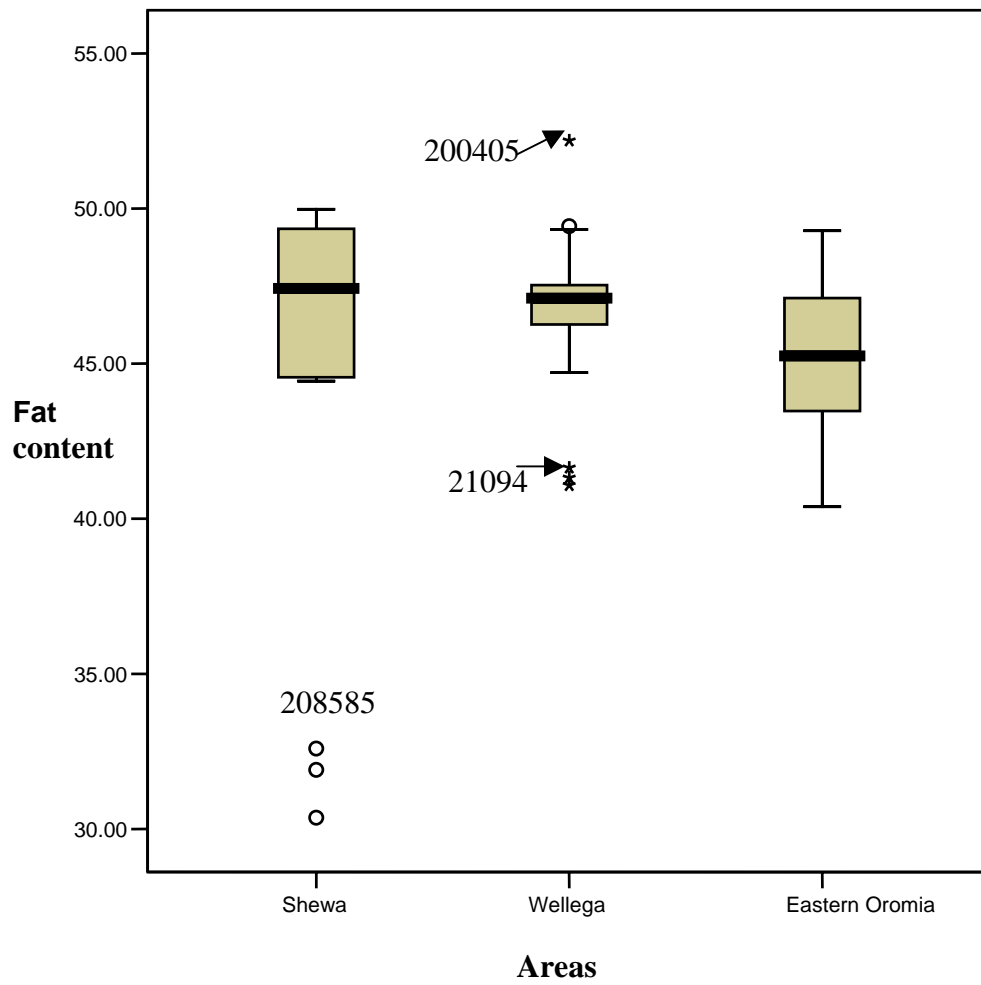


Fig. 5 The diversity of crude fat content in the seed samples of *B. carinata* populations from three areas.

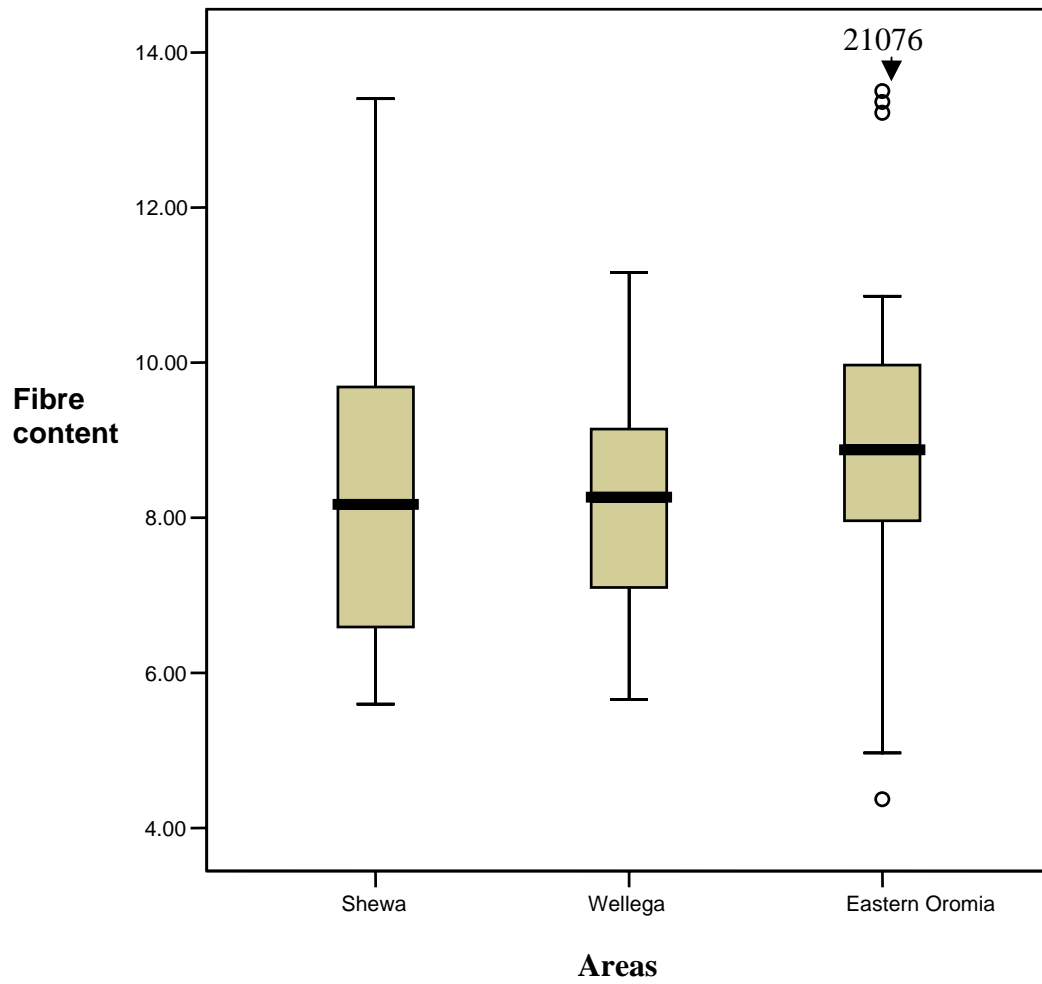


Fig. 6 The diversity of fibre content in the seed samples of *B. carinata* populations from three areas.

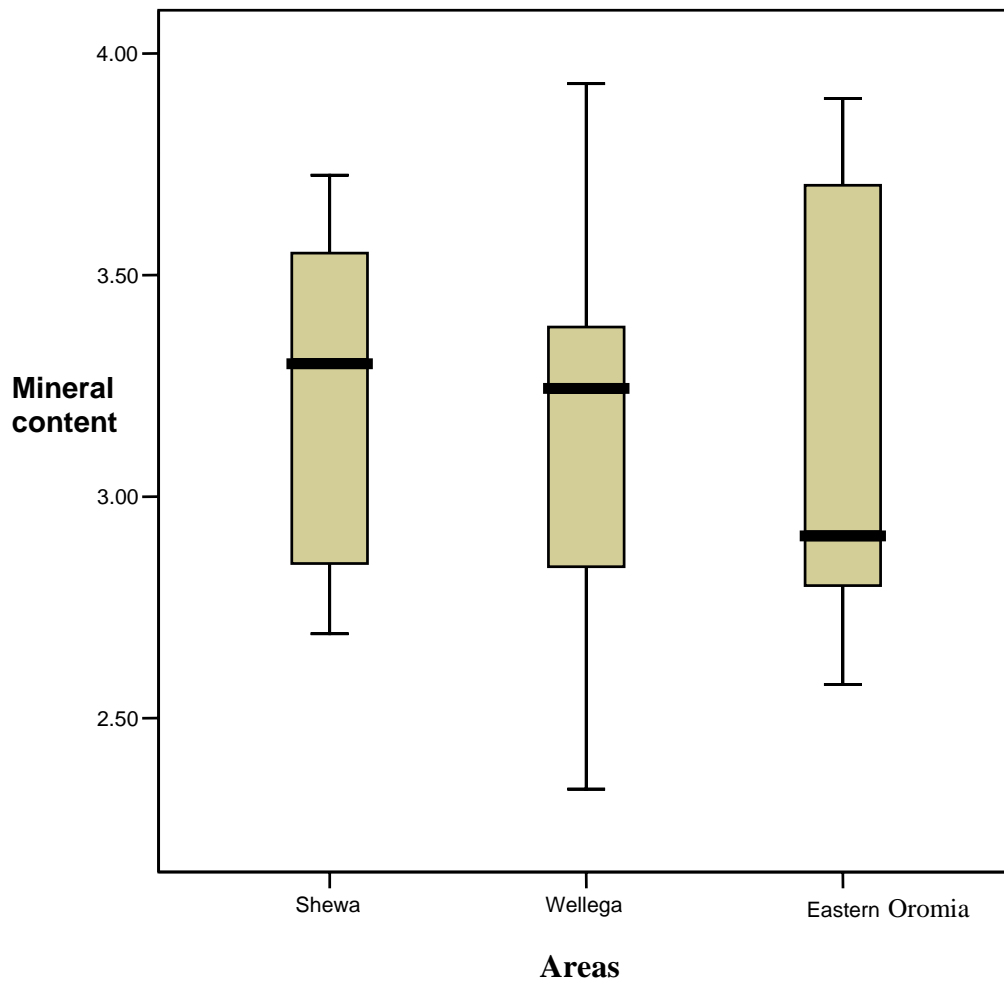


Fig. 7 The diversity of mineral content in the seed samples of *B. carinata* populations from three areas.

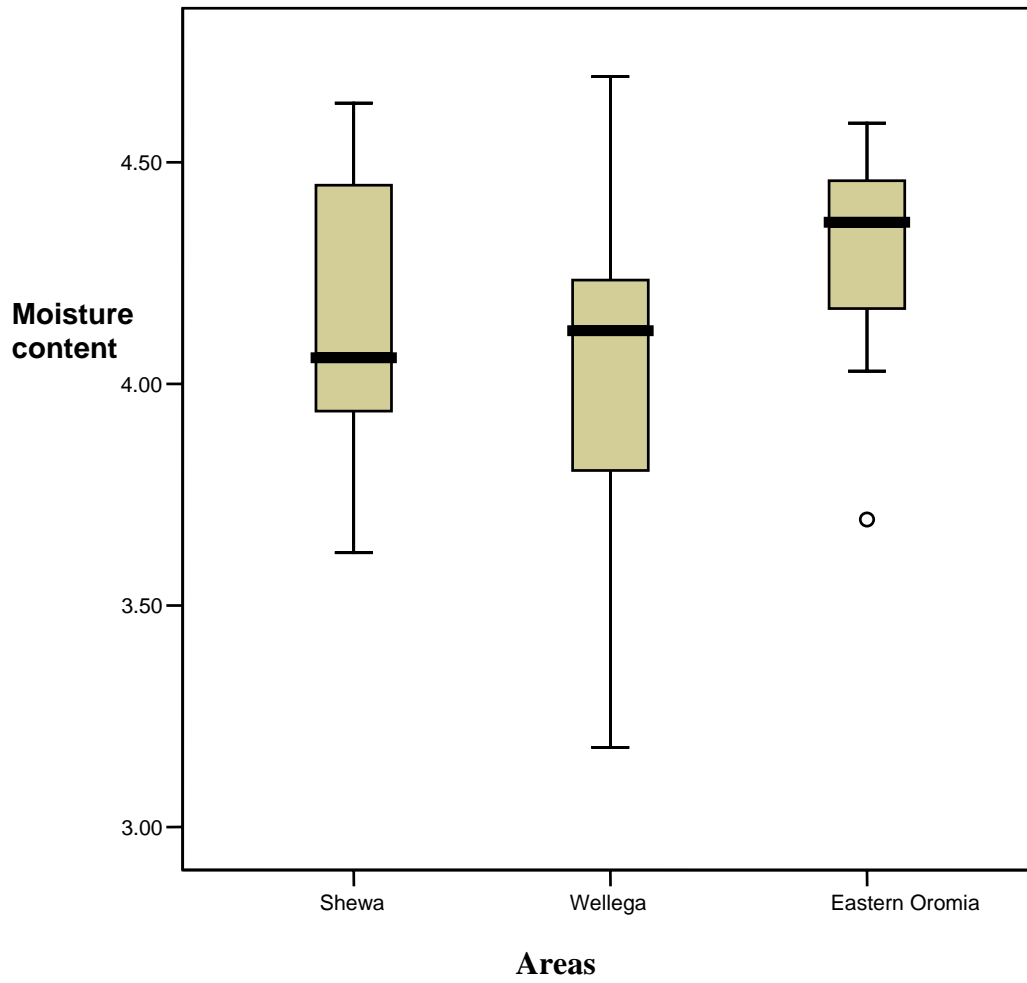


Fig. 8 The diversity of moisture content in the seed samples of *B. carinata* populations from three areas.

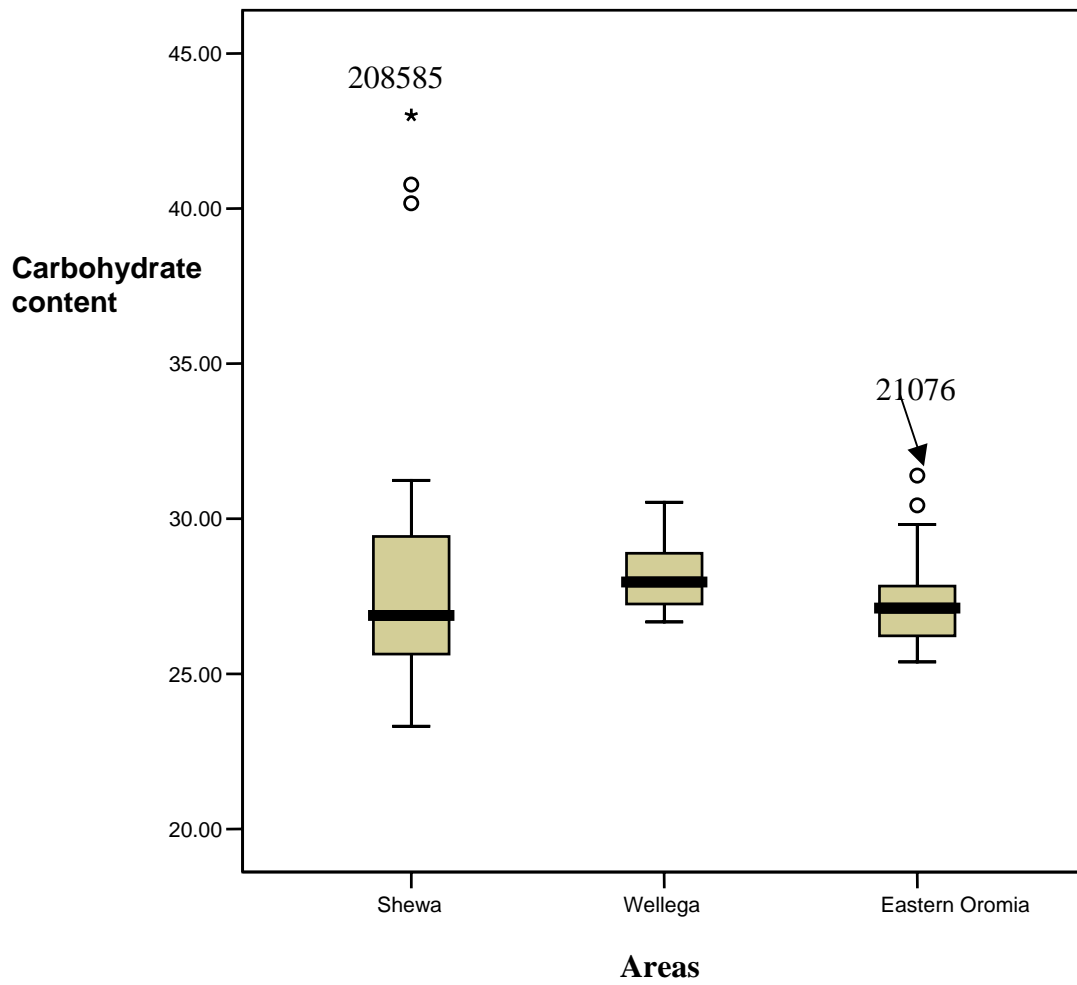


Fig. 9 The diversity of carbohydrate content in the seed samples of *B. carinata* populations from three areas.

6.3 Correlation Analysis

It was observed that protein content of seeds of *B. carinata* and *B. napus* samples under consideration was highly and negatively correlated with that of crude fat content at $P \leq 0.01$ level. Furthermore, the protein content was strongly correlated with that of moisture content of the samples at $P \leq 0.01$ but only in an opposite direction to fat content. The crude fat content analyzed showed strong negative correlation with that of crude fibre and carbohydrate content at $P \leq 0.01$ level. The fibre and carbohydrate contents also were correlated positively at $P \leq 0.05$. Mineral ash contents of the seeds have shown positive correlation with that of moisture content at $P \leq 0.05$. Moreover, the moisture content was correlated negatively with that of carbohydrate content at $P \leq 0.05$ level.

Table 5 Correlation coefficient matrix between the six proximate compositions of seeds used in the study.

		protein	Fat	Fibre	Mineral	Moisture	Carbohydrate
Protein	Pearson Correlation	1					
	Sig. (2-tailed)						
	N	81					
Fat	Pearson Correlation	-.529(**)	1				
	Sig. (2-tailed)	.000					
	N	81	81				
Fibre	Pearson Correlation	.157	-.285(**)	1			
	Sig. (2-tailed)	.160	.010				
	N	81	81	81			
Mineral	Pearson Correlation	-.188	-.076	.026	1		
	Sig. (2-tailed)	.093	.673	.820			
	N	81	81	81	81		
Moisture	Pearson Correlation	.307(**)	.076	.070	.265(*)	1	
	Sig. (2-tailed)	.005	.502	.535	.017		
	N	81	81	81	81	81	
carbohydrate	Pearson Correlation	-.015	-.825(**)	.221(*)	-.095	-.246(*)	1
	Sig. (2-tailed)	.895	.000	.048	.398	.027	
	N	81	81	81	81	81	81

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

6.4 Principal Component Analysis (PCA)

In the PCA, six variables were obtained from the six parameters measured in the study. Among these, three components were chosen. These are components with Eigen values greater than one. These three components explained around 80 % of the variation among the samples.

Table 6 Result of principal component analysis of *Brassica* oilseeds

Component	Eigen values					
	Total	% of Variance	Cumulative%	Total	%of Variance	Cumulative %
1	2.235	37.252	37.252	2.235	37.252	37.252
2	1.451	24.191	61.443	1.451	24.191	61.443
3	1.057	17.612	79.059	1.057	17.612	79.059
4	0.799	13.314	92.370			
5	0.458	7.630	100.000			
6	9.571E-11	1.595E-09	100.000			

Table 7 Component scores of the coefficient matrix.

Proximate Composition	Principal component		
	1	2	3
Protein content	0.256	0.378	-0.472
Fat >>	-0.454	-0.024	-0.066
Fibre >>	0.222	0.107	0.238
Mineral >>	-0.085	0.269	0.736
Moisture >>	0.012	0.607	0.058
Carbohydrate >>	0.376	-0.307	0.267

The first principal component (PCO 1) explained about 37.25 % of the total variability. In this principal component, crude fat content has a significant negative loading, carbohydrate and protein contents of the seeds have a significant loading but in the opposite direction to that of fat content of the seeds. The second principal component (PCO 2) explained about 24.19 % of the variability. In this component crude protein and moisture contents have a significant positive loading and carbohydrate content loads significantly in the opposite direction to them. Finally, the third principal component (PCO 3) explained 17.6 % of the variability. In this component mineral ash content loads significantly in the positive direction and, protein content loaded significantly in the opposite direction to the mineral content.

Furthermore, the PCOs were used to see the distribution of the samples in relation to components. In relation to the first and second PCOs, most samples aggregated together. In this ordination, few samples showed diversion from the general cluster analysis. These are acc. No 208585(1), 200405(17), 21076(20), 21094(12). Again, based on the second and third PCOs, the samples revealed close clustering. But, here, the clustering is more dispersed than the first one. Here, also acc. No 200405 has allocated it self away from the general grouping again. The final ordination of the samples (PCO 1 against PCO 3) similar phenomenon was also observed. In this distribution, acc. No 21076, 21094, 212224(13) and 208611(10) were also away from the general close clustering of the samples. Generally, most samples aggregated together with exceptionally few samples diverted away from the major aggregate in the ordination. An important observation here is that in the distribution of samples, samples that were away from the general aggregate were similar with those samples that were distinctly placed in the within diversity analysis of the box plots.

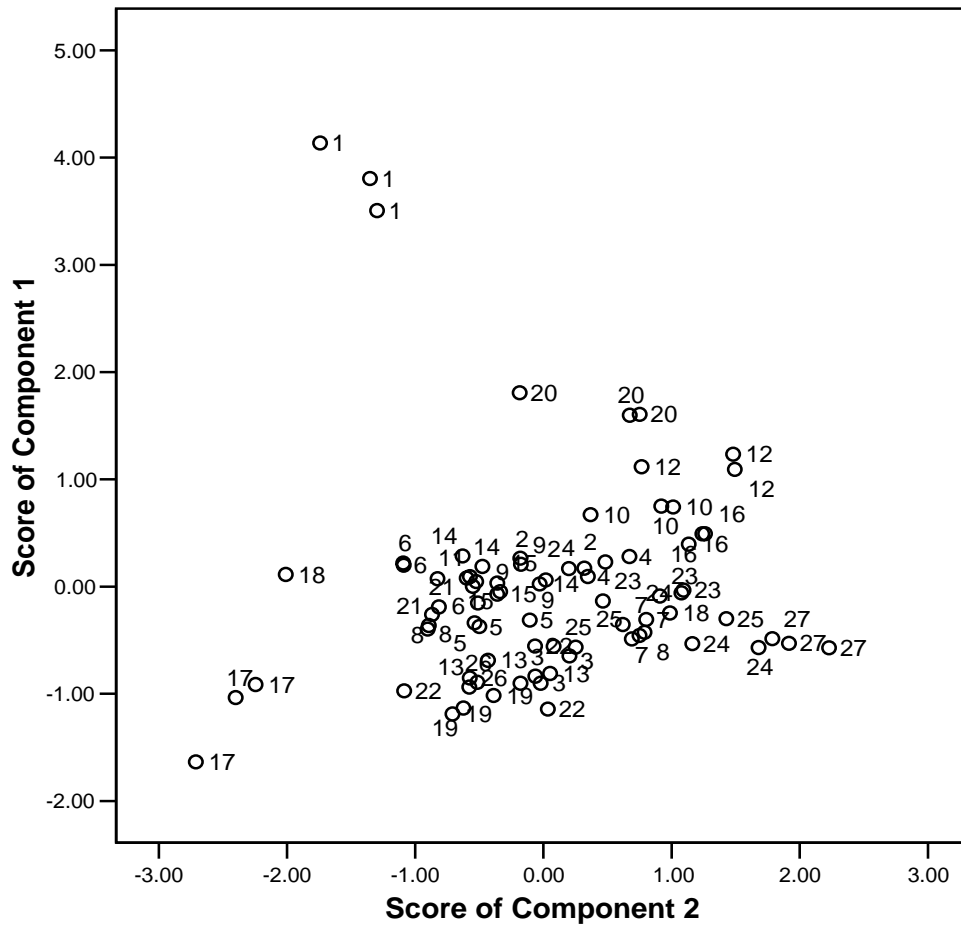


Fig. 10 The distribution of populations and cultivars of Ethiopian Mustard (*B. carinata*) and introduced cultivars of *B. napus* in relation to the first and second principal components.

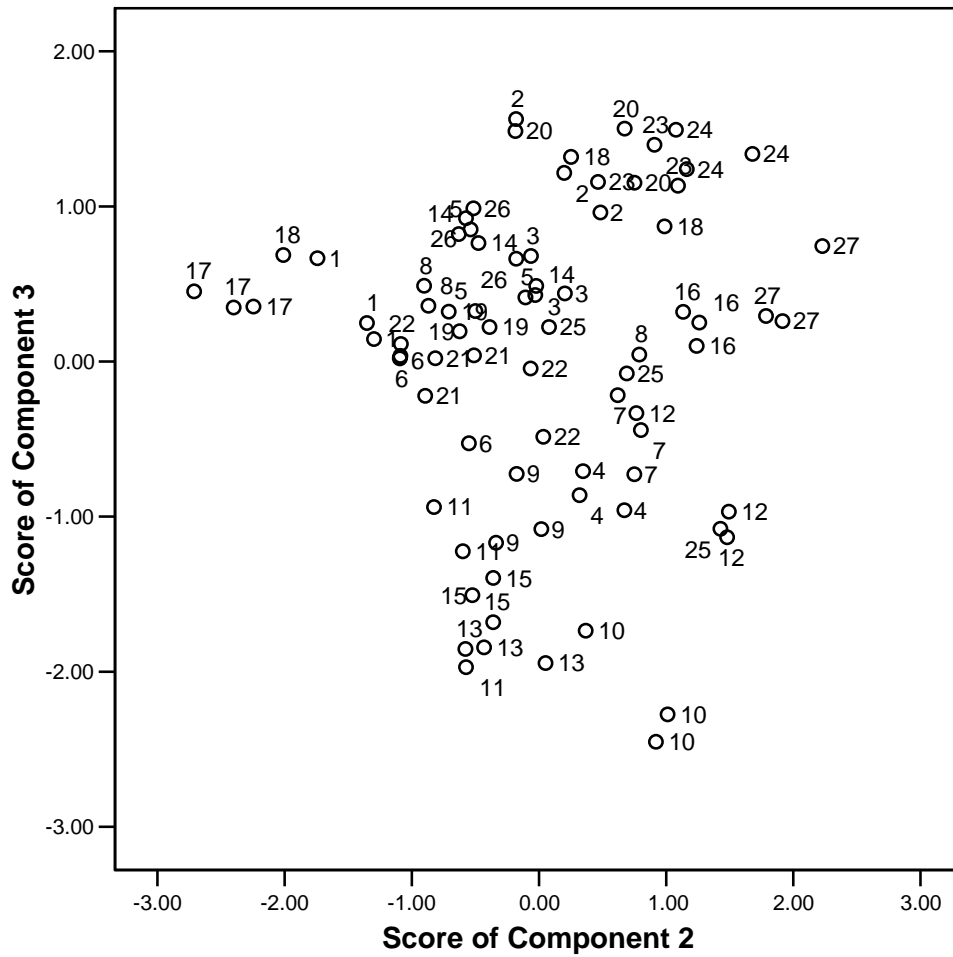


Fig. 11 The distribution of populations and cultivars of Ethiopian Mustard (*B. carinata*) and introduced cultivars of *B. napus* in relation to the second and third principal components.

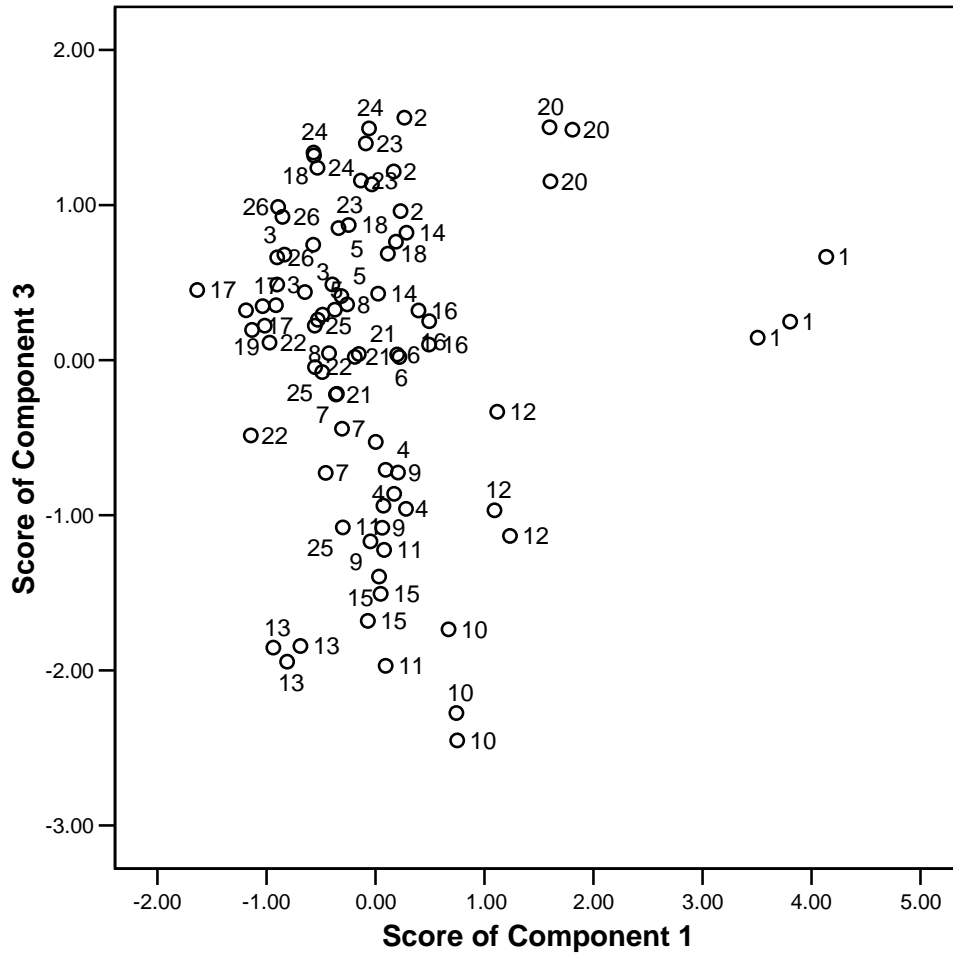


Fig. 12 The distribution of populations and cultivars of Ethiopian Mustard (*B. carinata*) and introduced cultivars of *B. napus* in relation to the first and third principal components.

6.5 Hierarchical cluster analysis

In the hierarchical cluster analysis, the seed samples clustered randomly. But, there is a general trend of grouping of the cultivars together; this implies that they are similar in most of their proximate compositions. Below 5.0 cut off value of Euclidean distance, many clusters were formed. Among these clusters, one cluster is dominated by cultivar seed samples.

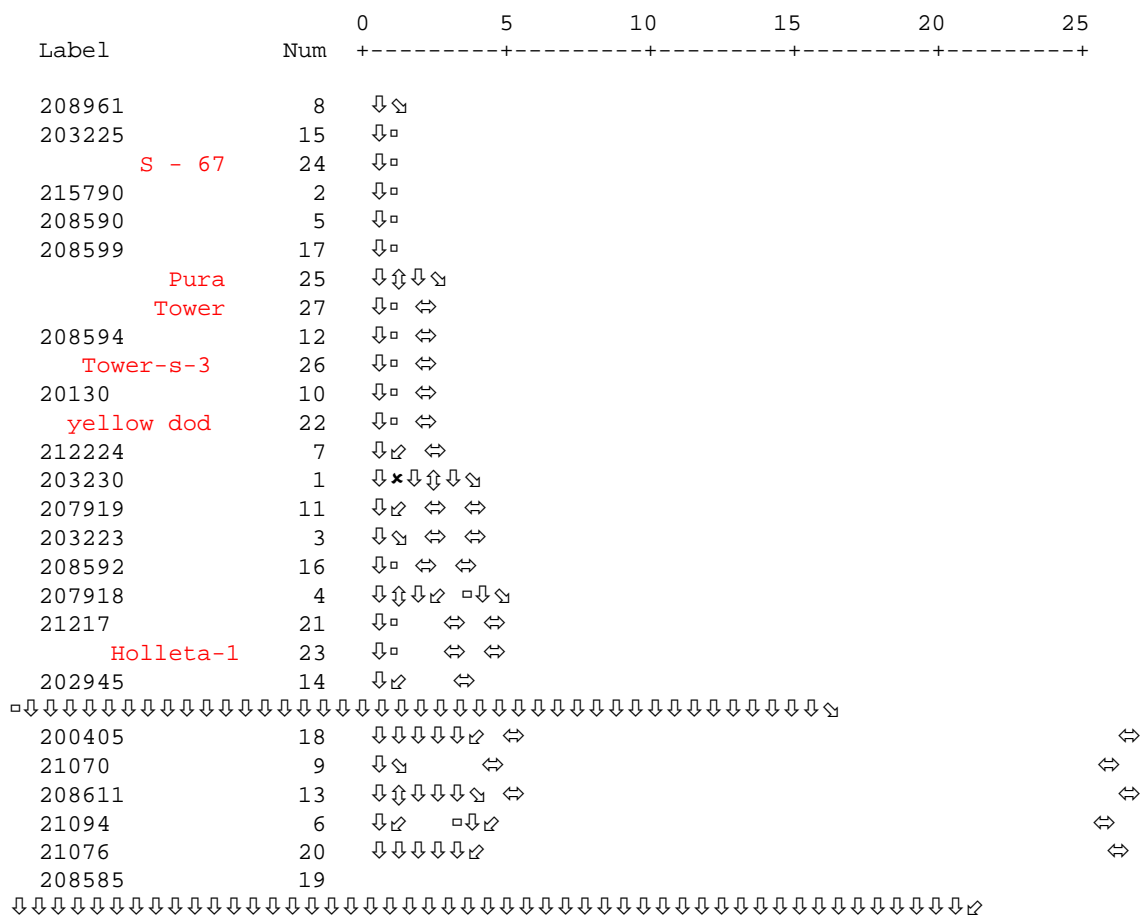


Fig. 13 Dendrogram based on Euclidean distance for all six proximate composition of the seed samples of the populations and cultivars.

7. DISCUSSIONS

7.1 Variability in Composition of Seed Quality Traits of *B. carinata* populations from different Areas and Altitude Classes

One way ANOVA was carried out to see if there is a significant variation between the three areas in proximate composition of seed samples of *B. carinata* populations. Thus, in the analysis significant variation was obtained only for protein and moisture contents of the seed quality traits. For these two quality traits, to see where the variation lies Post Hoc test was carried out furthermore. From the test, it was learned that the protein content of seed samples from Eastern Oromia populations have got higher mean protein content than the populations from other two areas. Therefore, this result implies, priority should be given to Eastern Oromia populations in order to make core collection for higher protein content of the populations of *B. carinata*. Furthermore, concerning moisture content, populations from the Wellega and Shewa areas were less than that of populations from Eastern Oromia. But, significant variation was obtained only between Wellega and Shewa seed samples. Thus, the seed samples from the Eastern Oromia showed higher protein and moisture contents, which agrees with the correlation analysis result that states these two proximate compositions are positively correlated. Therefore, this ANOVA result agrees with that of the correlation analysis carried out in this study. For the rest of the seed proximate compositions the between group variation analysis revealed no significant variation exists between the areas in the study.

In addition to the between localities ANOVA analysis, population were compared based on their altitude classes regardless of their origin. In the analysis, significant variation was obtained only for protein, fibre and carbohydrate contents of the seeds. In general, in both cases of between group analyses (region of origin and altitude classes) significant variation was obtained only for few proximate compositions. Therefore, this implies the fact that agro-ecology has a limited effect on seed quality traits of *B. carinata*.

7.2 Diversity in Seed Quality Traits of *B. carinata* within Each Area

The overall diversity for six seed quality traits was examined in each area. In general, the analysis revealed significant variation within each area. In the case of crude protein content, seed samples from the Wellega region showed the highest diversity. The highest and the lowest protein content of all seed samples were also found in samples of this locality. The average protein content of all samples (18.66 %) was lower than the average protein content of seeds samples (30.1 %) reported from all over the country (Alemayehu and Becker, 2002). This reduction of crude protein content may be due to the reduced sample size and sample area cover in the present study. Moreover, in the present study, it was found that the lowest protein content of samples from Eastern Oromia was higher than the highest protein content of Wellega and was comparable to that of the highest protein content recorded for seed samples of Shewa. Thus, regarding crude protein content, population from Eastern Oromia should be considered thoroughly.

B. carinata is an oil crop, thus its seeds are of great importance than its vegetative parts. Previous studies on the seeds of *B. carinata* grown at Holleta, showed an oil content range from 16.4 % to 54.7 % with a mean of 32.3 % (Teklewold, 2005). In the present study, highest diversity was observed in the seed samples of Shewa area with a range value from 30.36 % to 49.97 %. Therefore, the range obtained in the present study is narrower compared to that of the previous, which may be due to the limited number of samples in the study. Nevertheless, in the present study, the highest fat content was from Shewa region and the lowest was from Eastern Oromia. Furthermore, the diversity of the fat content of seeds from Wellega was very much lower, which in turn lead to narrow protein content diversity, since they are negatively correlated.

In previous studies on *B. carinata* seed samples, the crude fat content was further analyzed. In this analysis, the major proportion of the total fatty acid content was contributed by erucic acid. But, it also contains significant amount of linoleic acid which is nutritionally beneficial in oilseeds vegetables (Teklewold, 2005). Linoleic acid helps to prevent cardiovascular disorders such as coronary heart diseases and high blood pressure (Dagne

and Jonsson, 1997). Thus, if the erucic acid content can be reduced from and linoleic acid can be increased in *B. carinata*, the importance of this indigenous crop will be remarkable.

Comparison of samples from the different areas for their fibre content showed significant variation. Nevertheless, the highest diversity was observed in Shewa seed samples. But, regardless of the high diversity in Shewa populations, the highest fibre content belongs to seeds from the Eastern Oromia populations. One unique observation of the fibre content diversity analysis in Eastern Oromia seed samples was, acc. No. 21076 showed a significantly higher amount of fibre content. Thus, this population could serve as good source of dietary fibre, which may improve bowel functioning and reduce plasma cholesterol (Enjiugha and Ayodele-Oni, 2003). In the case of mineral content, highest diversity was observed in populations from Wellega area. Moreover, the seed samples from this locality include the lowest and highest mineral content of the samples analyzed. Here, in the case of mineral content the most samples revealed relative similarity.

For moisture content of *B. carinata* populations, the seed samples from Wellega region showed the highest diversity. In the case of moisture content there is strong similarity in all of the seed samples which indicate absence of significant genetic differentiation at loci controlling the trait. Since moisture content is correlated with protein content, thorough emphasis should be given to moisture content of the seed samples. The carbohydrate content of the seed samples was also observed and it revealed significant variation exists between the areas considered. However, the highest diversity was observed in Shewa region. In this area, acc. No 208585 has got the highest carbohydrate content which is significantly higher than all the seeds included in the study. However, its fat content was the least among all the samples. Therefore, this diversity analysis also agrees with that of the correlation analysis result, which states carbohydrate and fat contents of *B. carinata* seeds are negatively correlated.

In general, the three areas under study revealed different type of seed quality traits diversity. Especially, the seed samples with highest value of protein content in each of the

area have the least fat/oil content. Thus, this within diversity analysis result agrees with the correlation analysis of the study and is consistence with reports made by Teklewold (2005) on *Brassica* oilseeds. Nevertheless, those regions that showed higher diversity need to be explored further in order to collect and conserve the crop and make use of it by improving the quality and productivity of Ethiopian mustard.

7.3 Comparison between Released Cultivars and Populations of *B. carinata*

The released varieties of *B. carinata* in Ethiopia were compared with that of the populations from all localities included in the study. In the comparison, it was learned that the cultivars were relatively higher than some of the populations in terms of the quality parameters measured. However, the study revealed that there are some populations with better proximate compositions of some or most of the proximates as compared to released cultivars. These samples had originated from different areas under study. For example, acc. No. 208611 from Eastern Oromia has better oil and protein contents compared with that of the three released cultivars developed locally. Moreover, there are also seeds from other areas with equal or better seed quality traits. For example, acc. No. 203225 and 212224 from Shewa and 208961 from Wellega could be mentioned with higher seed proximate composition of one or more of the seed quality traits studied. Thus, keeping the other agronomic qualities that the released cultivars have got, there should be a further enhancement program aimed at upgrading the proximate composition by selection and/or hybridization.

In addition, when each of the three released cultivars of *B. carinata* was compared with each other, all of them turned to be of similar proximate composition. Therefore, in addition to other agronomic qualities that the cultivars have got, all of them should be a primary choice of the farmers than population accessions, to take advantage of the enhanced seed quality trait at hand.

Furthermore, three introduced varieties of *B. napus* were included in the study. These introduced cultivars showed relatively higher amount of oil content than that of the varieties developed locally even though they are of different species. Despite the fact that oil and protein content of the introduced cultivars was better than the cultivars in Ethiopia, the study revealed that there are population with equal or even better seed proximate composition compared to them. Therefore, it can be learned that there are genes of importance in the introduced *B. napus* varieties which can be introgressed to the local varieties via hybridization for seed quality trait improvement. However, similar genes with desirable effect for proximate composition can be obtained from the local landrace populations. Therefore, this study showed that the Mustard landraces in Ethiopia are performing as equal as or better than that of introduced cultivars and hence breeders need to exhaust already existing collections in the country, before going to the introduction of any cultivars.

When the other seed quality traits, like mineral and moisture contents were considered populations from different localities and the cultivars were comparatively similar. However, there are populations with higher mean value of carbohydrate content than that of the cultivars. For example acc. No, 202945, 21076, and 200405 etc are the ones to be mentioned. Furthermore, for the fibre content similar phenomenon is expected, since it is positively correlated with that of carbohydrate content. Nevertheless, even though the mineral, fibre and moisture contents of the seeds under consideration are in lesser amounts than the other proximates, enough consideration should be given for those seed quality traits since they are correlated with rest in one way or the other.

7.4 Correlation Analysis among the Six Proximate Compositions of *B. carinata*

Correlations among traits are useful for selecting genotypes possessing group of desired characters. However, such correlation coefficients could vary with genotypes studied and the environments where the test is carried out (Hadjichristoulou, 1987). In the present study, the direction of correlations between crude fat/oil content and that of crude protein

content among the samples under consideration was negative. This correlation result was also obtained by previous studies on *B. carinata* (Alemayehu, 2001; Teklewold, 2005)

Quantitative trait loci (QTL) mapping studies carried on *B. napus* gave evidence that the oil and protein contents of the seeds of *B. napus* are controlled by genes with both pleiotropic and linked effect (Zhao, 2002). Therefore, as the *B. carinata* and *B. napus* are closely related in their genetic basis, similar gene effect is expected to control the protein and oil contents of *B. carinata* seeds. Thus, search for seeds of *B. carinata* with better oil or protein content, will have a direct opposite influence on the other seed quality trait. Therefore, a joint improvement technique of both oil and protein contents should be carried out by selecting those seeds with higher amounts of both seed quality traits instead of either. The rationale behind this approach is to obtain more oil and protein contents as a result of more effective partitioning of the available photosynthate in to intrinsically more valuable products (Röbbelen and Thies1980a). Furthermore, the populations with high protein or fat content can be selected depending on interest of breeders or consumers regardless of the negative correlation between the two seed quality traits (Teklewold, 2005).

Another important observation revealed strong negative correlation between crude fat and carbohydrate content of the seeds of *B. carinata*. Furthermore, the fat content of the seeds is negatively correlated with that of fibre content. This is disadvantageous for breeders and consumers from the point of view that those seeds with high fat/oil content are negatively correlated with other important seed quality traits (protein, carbohydrate and fibre).

In this correlation analysis there are also other correlations results observed between the traits measured in the study. In general, this correlation matrix results implies the fact that care should be taken in future attempts in seed quality traits of *B. carinata* populations. Nevertheless, knowledge of the correlation coefficients within the seed of the crops is beneficial for breeders to select better quality seeds for future breeding and for end-users like milling oil companies (Maria *et al.*, 2004).

7.5 Distribution of Samples of *Brassica* Oil seeds with Reference to the three Principal components

Principal component analysis (PCA) is a data analysis tool that is usually used to reduce the dimensionally large number of interrelated variables. Three dominant groups of variables were selected by reducing the number of variables in this study. But, it can be argued that one component should be extracted from each variable included in the study (Tsige *et al.*, 2005b).

In the principal component analysis, most of the seed samples with high protein and carbohydrate contents dominated the first principal component in the positive direction. In the second principal component scores, those seeds with high protein content dominated the component positively. Whereas, in the third principal component; samples with high mineral ash content loaded positively. In general, what a positive loading indicates is that there is a positive correlation between the component scores and the variables (Tsige *et al.*, 2005b). Here, in all of the three principal components the protein content of the seed samples loaded significantly either in the positive or negative direction. This indicates, protein content of the seeds had contributed a lot for the variation observed in the study.

Furthermore, the distribution of seed samples based on their six seed quality traits, revealed absence of aggregation of the samples due to place of origin or altitude classes. However it was noticed that there are some populations which have aggregated away from the general grouping. These populations should be given more emphasis by breeders in the future studies. By doing so, it is possible to take advantage of their uniqueness and deviations. Nevertheless, the absence of grouping of the seeds samples based on origin or altitude implies that the lesser impact is imposed by environmental factors on seed quality traits of *B. carinata*.

7.6 Cluster Analysis and its implication to the improvement of Ethiopian Mustard

The cluster analysis, revealed the random placing of the samples on the dendrogram. This implies the fact that seeds within the cluster failed to group based on their place of origin or adaptation zone. However, detailed analysis with more samples on these parameters should be examined, prior to this conclusion. Moreover, the absence of aggregation of seed samples of similar origin and altitude class in the same group implies the fact that there might be possible seed flow via market based seed exchange or more specifically gene flow among the area in the study.

In the hierarchical cluster analysis based on the six seed quality traits, below 5.0 cut-value of Euclidean distance, seven clusters were formed. The first cluster included five of the six cultivars involved in the study. These varieties are Yellow-Dodolla, S-67, Pura, Tower-S-3 and Tower. The remaining cultivar, Holleta-1, did not cluster with rest. This departure may be due to the *B. juncea* background in its genome make up during the development of the cultivar. Moreover, clustering of S-67 and Yellow-Dodolla with that of introduced cultivars from abroad implies that they are of good seed quality traits.

Furthermore, as the grouping is based on similarity in seed quality traits, more emphasis should be given for those population accessions which were clustered with that of the varieties. In addition, all of the cultivars of *B. napus* were clustered in the same group, which indicates presence of consistency for the six seed quality traits under consideration. This may be due to the fact that they are phylogenetically similar or contain similar genes for the seed quality traits used in the study.

8. Conclusion and Implications for Breeding

Variability in seed quality traits of populations of *B. carinata* and cultivars of *B. carinata* and *B. napus* was studied by using proximate analysis. The analysis was performed according to the Official methods of analysis by AOAC international.

The analysis revealed the presence of significant variation in the seed quality traits within the areas considered and between them. Furthermore, significant variation was also obtained for the between altitude classes comparison. However, when variation among the areas was analyzed; only few proximate compositions showed significant variation. In addition, the samples from different altitude classes regardless of their place of origin showed significant variation for some of the seed quality traits. But, the variation within areas was significant for all the traits under consideration. In general, there is no clear pattern observed i.e. populations from different areas and altitude classes dominated the diversity observed, the highest and lowest value of the proximate analysis. Furthermore, this study showed the absence of a population or cultivar with all the desired seed quality traits considered in study. Therefore, this implies the fact that selection alone is not good enough to enhance the seed quality traits *B. carinata*.

In the correlation analysis, various relationships between the six seed quality traits were observed. In this correlation analysis, negative correlation was observed between crude protein and crude fat/oil contents of the seed samples. This negative correlation result is in line with other studies carried on *Brassica* oilseeds. Moreover, the study has also investigated correlations between the other proximate compositions which were not included in previous studies on *B. carinata*.

The principal component analysis gave rise to three dominant components explaining nearly 80% of the variability. In this analysis, the distribution of the samples in relation to the three components was not influenced by the origin or altitudinal classes of the samples; rather their seed quality traits determined the distribution.

Comparison between the nationally released cultivars of *B. carinata* and that of the populations indicated that the released varieties have more or less improved proximate composition. However, the study revealed that there are some population accessions from different localities with better seed quality traits for any of the proximate compositions. This study has provided specific information for breeders working on enhancing *B. carinata* proximate composition. Thus, by enhancing the quality of the crop it might be possible increase the interest of milling companies and/or farmers to cultivate *B. carinata* as a main crop.

In general, previous studies on *B. carinata* seeds were focused only on the oil and protein contents of the seeds. However, this study has also generated information on the other quality traits of the seeds. These are carbohydrate, mineral, fibre and moisture content of the seed. Therefore, the study can serve as a benchmark for future studies on seed chemistry of *B. carinata*.

9. Recommendations

A financial issue has limited the number of *B. carinata* and *B. napus* seed samples used in the present study. Therefore, similar studies of large sample size covering the whole country is required to select parents of desired proximate composition and put these selected seeds through further hybridization program for improvement of these and other agronomic traits.

In future studies, knowledge obtained from clustering of populations of *B. carinata* in the present study could be used as a benchmark for future collection and characterization of landraces. Furthermore, molecular level characterizations need to be done in order to make comparison with present study and further determine the existing diversity at the gene level.

The study had also revealed the presence of diversity, which needs to be explored further. This was indicated by the fact that some populations of *B. carinata* were better than the released varieties in their proximate composition. In general, the study implied the importance of intensive collection and characterization work needed to be done in the future.

10. References

- Abebe, D., Dawit, T., Getahun, M. and Debitu, B. (1992). Ethiopia's genetic resources of oilseeds. **In:** *proc. 1st national oilseeds workshop*. Institute of Agricultural Research, Addis Ababa, Ethiopia. 12 - 17 pp.
- Ahmed, H., Hasnain, S., and Khan, A. (2002). Evolution of genomes and genome relationships among the rapeseed and mustard. *Biotechn.* **1**: 78 - 87.
- Alemayehu, N. (2001). Germplasm diversity and genetics of quality and agronomic traits in Ethiopia mustard (*Brassica carinata* A. Braun). PhD Thesis, George August University of Gottengen, Germany, 127 pp.
- Alemayehu, N. and Becker, H.C. (2002). Genotypic diversity and pattern of variation in a germplasm material of Ethiopian mustard (*Brassica carinata* A. Braun). *Genet. resour. crop evol.* **49**: 573 - 582.
- Andargie, A. (2006). Genetic diversity within and among accessions of Ethiopian Mustard (*B. carinata* A. Braun) collections from south Gonder, Arsi and Tigray areas of Ethiopia using Allozyme analysis. Addis Ababa University, Ethiopia.
- AOAC International (1996). Official Methods of Analysis. **In:** *AOAC International*, 16th ed. (Cunniff, P. ed). AOAC International Suite 500, Maryland.
- Arunachalam, V. (1981). Genetic distance in plant breeding. *Indian J. Genet.* **41**: 226 - 236.
- Asfaw, Z. (1992). Conservation and use of traditional vegetables in Ethiopia. **In:** *Traditional African vegetables. Proceedings of IPGRI Workshop on Genetic Resources of Traditional \Vegetables in Africa. Conservation and Use* Guarino, L. (ed) 29-31 August 1995, TCRAF-HQ, Nairobi, Kenya. 57 - 63 pp.
- AVRDC Fact sheet (2003). *Brassica carinata*. AVRDC International cooperators fact sheet.
- Bayeh, M. and Gebre Medhin, T. 1992. Insect pests of Noug, linseed and *Brassica* in Ethiopia. **In:** *Oilseeds research and development in Ethiopia, Proceedings of the first National Oilseeds Workshop, 3-5 December 1991*. Addis Ababa, Ethiopia. 174 - 178 pp.

- Becker, H.C., Loiptien, H. and Röbbellen, G. (1999). Breeding of the *Brassica*: An overview. **In: *Biology of Brassica coenospecies***. Gomez-Campo, C. (ed). Elsevier, science BV, Amsterdam, 413 - 460 pp.
- Bekele, E. (1985). The biology of cereal landrace populations: problems of gene conservation, plant breeding selection schemes and sample size requirements. *Hereditals* **103**: 119 - 134.
- Bhatt, G.M. (1970) Multivariate analysis approach to selection of parents for hybridization naming at yield improvement in self-pollinated crops. *Aust. J. Agric. Res.* **21**: 1 - 7.
- BioMatnet (2006). *Brassica carinata*: The outset of a new crop for biomass and industrial non-food oil. Fair. CT96 - 1946. Updated by CPC press: 08/10/2006.
- Brassicaceae (2006). **In: Encyclopedia**. Retrieved December, 3 2006, from Encyclopedia Britannica online; [http:// www:britannica. Com / eb/ article 9016252](http://www.britanica.com/eb/article/9016252).
- Breitenbatch, V. (1963). The Indigenous Trees of Ethiopia *2nd ed.* Ethiopian Forest Association, Addis Ababa, Ethiopia, 303 pp.
- Cardone, M., Prati, M.V., Rocco, V., Seggiani, M., Senatore, A. and Vitolo, S. (2002). *Brassica carinata* as an alternative oil crop for the production of bio-diesel in Italy: engine performance and regulated and unregulated exhaust emissions. *Env. Sci Tech.* **36**: 4656 - 4662.
- Chen, B., Hanseen, W., and Simonsen, V. (1989b). Isozyme studies in *Brassica* species. **In: *Inheritance and linkage relationship of Isozyme loci in Brassica campestris L. Genome***. PhD. Thesis, Swedish University of Agricultural Science, Sweden, 220 pp.
- Chen, B.Y., Haseen, W.K and Simonsen, V. (1989a). Comparative and genetic studies of Isozymes in re-synthesized and cultivated *Brassica napus* L., *B. campestris*, L. and *B. alboblabra* Bailey. *Threor. Appl. Genet.* **76**: 00 - 00.
- CSA (Central Statistics Authority) (2003). Agricultural sample survey 2002/. Report on area and production for major crops. Statistical bulletin 200, Addis Ababa. Ethiopia, 96 pp.

- Dagne, K. and Jonsson, A. (1997). Oil content and fatty acid composition of Seeds of *Guizotia* Cass (Compositae). *J. Sci. Food Agric.* **73**: 274 - 278.
- De Haro, A., Dominguez, J., Garcia-Ruiz, R., Velasco, L., Del Rio, M., Munoz, J. and Fernandez-Martinez, J. (1998). Registration of six Ethiopian mustard germplasm lines. *Crop Sci.* **38**: 558 - 561.
- Dessalegne, L., Herath, E., Belehun, B., Lemaga, L.G. and Mariam, S. (1994). Horticultural Research and Development in Ethiopia: Proceedings of the 2nd National Horticultural Workshop of Ethiopia, IAR, 1992, IAR, Addis Ababa, 19 - 36 pp.
- Downey, R. (1996). *Brassica* Oilseed breeding: achievements and opportunities. *Plant Breed.* **60**: 1165 - 1170.
- Edwards, S., Tadesse, M., Demissew, S. and Hedberg, I. (2000). *Flora of Ethiopia and Eritrea*. macnoliaceae to flacourtiaceae. (2). National Herbarium, Addis Ababa, Ethiopia and Uppsala, Sweden, 121 - 125pp.
- Ennujiugha, V.N. and Ayodele-Oni, O. (2003) Evaluation of nutrients and some anti-nutrients in lesser-known, underutilized oilseeds. *Inter. J. food sci. Tech.* **38**: 525 - 528.
- Ferris, R.A., Flores, R.A., Shanklin, C.W., and King, M.K. (1995). A proximate analysis of food service waste. *Appl. Engin. Agri.* **11**: 567 - 572.
- Food and Agricultural Organization of the United Nations (FAO) (2002). *Brassica carinata* [<http://ecoport.org/epplant>] 01, 29, 2002: 16: G. M. T.
- Food and Agriculture Organization of the United Nations (FAO) (1988). Report of the Regional Expert consultation on the Asian Network for food and Nutrition, Bangkok. May 3-6, Regional office for food and the pacific (RAPA).
- Getinet, A., Rakow, G. and Downey, R. (1996). Agronomic performance and seed quality of Ethiopian mustard in Saskatchewan. *Can. J. Plant Sci.* **76**:387 - 392.
- Getinet, A., Rakow, G. and Raney, J.P. (1996). Glucosinolate content variation in *Brassica carinata* A. Braun germplasm grown at Holleta Ethiopia. *Eucarpia Cruciferae Newsl* **18**: 84 - 85.

- Gomez-Campo, C. and Prakash, S. (1999). Origin and domestication of *Brassica*. **In:** *Biology of Brassica cenospecies*, Gomez-Campo, C., (ed.). Elsevier, Amsterdam. 33 - 55 pp.
- Graham, S.A., Hirsinge, F. and Röbbelen, G. (1981). Fatty acids of *Cuphea* (Lythraceae) seed lipids and their systematic significance. *Am. J. Bot.* **68**: 908 - 917.
- Gugel, R., Séguin-swartz, G. and Petrie, A. (1990). Pathogenicity of three isolates of *Leptosphaeria maculans* on *Brassica* species and other crucifers. *Can. J. Plant Pathol.* **12**: 75 - 82.
- Hadjichristoulou, A. (1987). Association between traits of chickpea varieties. *Intern. Chickpea News letter.* **17**: 2 - 4.
- Hawtin, G., Iwanaga, M. and Hodgkin, T. (1997). Genetic resources in breeding for adaptation. **In:** *Adaptation in plant breeding*. Tigerstedt PMA (ed) kluwer Academic publisher, Netherlands, 277 - 288 pp.
- Hemingway, J.S (1995). Mustards: *Brassica* spp. and *Snapsis alba* (Criciferae). **In:** *Evolution of crop plants*. 2nd ed Smart, J. and Simmonds, N. W. (eds). Longman Scientific and Technical, Singapore, 82 - 88 pp.
- Hirsinge, F. (1989). New annual oil crops. **In:** *Oil Crops of the World*. Röbbelen, G., Downey, R.K. and Ashri, A. (eds), McGraw-Hill, New York, 518 - 532 pp.
<http://www.agbios.com>. Nutrition Data. Retrieved on 18 Jun, 2007 23:05:02 GMT.
- IENICA (2004). Generic guidelines on the agronomy of selected industrial crops. Central Science Laboratory, Sand Hutton, New York.
<http://www.ienica.net/agronomyguide/agronomyguide05.pdf>. accessed retrieved on 4/13/2007.
- Institute of Agricultural Research (IAR) (1986). Highland oil crops. **In:** *Proceeding of the 18th National crop improvement conference*, Nazreth, Ethiopia. 259 pp.
- Institute of Biodiversity and Conservation (IBC) (2001). [Http://: www.Horticulture genetic/bioresearch@telecome.net.et](http://www.Horticulturegeneticbioresearch@telecome.net.et) October 25, 2006 16:30 GMT.

- Jain, S. and Singh, R. (1972) Population biology of *Avena*. II. Isoenzyme polymorphisms in population of the Mediterranean region and Central Europe. *Theor. Appl. Genet.* **41**: 79 - 84.
- Jain, S., Qualset, C., Bhatt., G., and Wu, K.K. (1975).. Geographical patterns of phenotypic diversity in a world collection of durum wheat. *Crop Sci.* **15**: 700 - 704.
- Jiang, C.X., Wright, R., El-Zik, K. and Paterson, A.H. (1998). Polyploid formation created unique avenues for responses to selection in *Gossypium* (cotton). *Proceeding of the National Academy of Science* **93**: 4419 - 4424.
- Koul, K.K., Ranja, N. and Raina, S.N. (2000). Seed coat micro sculpturing in *Brassica* and allied genera (subtribes Brassicaceae, Ruphaninae, Moricandiinae). *Ann. of Bot.* **86**: 385 - 397.
- Krik, R. and Sawyer, R. (1991). Pearson's composition and Analysis of foods, 9th. Longman scientific and Technical.UK, 708 pp.
- Kumar, D. (1995). Salt tolerance in oilseed *Brassicacae*: present status and future prospects, *Plant breed.* **65**: 1438 - 1447.
- Liu, X.P., Tu, J.X., Chen, B.Y. and Fu, T.D. (2003). Identification and inheritance of a partially dominant gene for yellow seed color in *Brassica napus*. *Plant breed.* **124**: 9 - 12.
- Maria, D.A., Eleni, S. and Haileselassie, Y. (2004). Isozyme variation and nutritional analysis in field pea (*Pisum sativum* L.) populations from Ethiopia. *Eth.J. Biol. Sci.* **3**: 133 - 151.
- Materson, J. (1994). Stomata size in fossil plants: evidence for polyploidy in majority of angiosperms. *Science* **264**: 421 - 424.
- Mazzoncini, M., Vannozzi, G.P., Megale, P., Secchiari, P., Pistioa, A. and Lazzai, L. (1993). Ethiopian Mustard (*Brassica carinata* A. Braun) crop in central Italy. Characterization and Agronomic evaluation. *Agr. Med.* **123**: 330 - 338.
- McVetty, P.M.E. and Scrath, R. (2002). Breeding for improved oil quality in *Brassica* speices. *J. Crop. Prod.* **5**: 345 - 369.

- Messele, T. (2001). Multidisciplinary approach in estimating genetic diversity of Ethiopian tetraploid Wheat (*Triticum turgidum* L.) landraces. PhD. Thesis, Wageningen University, Wageningen, the Netherlands, 108 pp.
- Mulligan, G. and Bailey, L.G (1976). Seed coat of some *Brassica* and *sinapis* meedy and cultivated in Canada. *Eco. Bot.* **30**: 143 - 148.
- Nei, M. (1978). *Molecular Evolutionary genetics*. Columbia University press, New York, USA 149 - 208 pp.
- Pandey, I., Singh, B. and Sachan, J. (1999). *Brassica* hybrid research in India: status and prospects. 10th rapeseed international congress. Pantanagar, India.
- Panoustou, C., Karadakis, G., and Namatou, E. (1999). *Brassica carinata*, a promising crop for non-food oil and biomass production in the Mediterranean regions. Biobase European Energy Crops interNetwork. Document ID Bio 464.
- Plant Genetic Resources (PGR) (1995). Ethiopia: Country Report to FAO International Technical Conference on Plant Genetic Resources. Addis Ababa, Ethiopia, 51 pp.
- Ragrumulu, N., Madhavan, N. and Kalyanasundaram, S. (1983). A manual of laboratory techniques. National institute of nutrition. Indian council of medical research, India. 359 pp.
- Rakow, G. (1995). Developments in the breeding of edible oil in other *Brassica* societies. In proc. 9th Int. Rapeseed Conf., Cambridge, Henry ling Ltd. Dochester, UK 410 - 412 pp.
- Ren, C. and Bewley, J. (1998). Seed development, testa structure and pre callus germination of Chinese cabbage (*Brassica rapa* subsp. *pekinensis*). *Seed Sci. Reas.* **8**: 385 - 397.
- Röbellen, G. (1981). Breeding for low content of glucosinolates in rapeseed. **In:** *World croos: Production and utilization of protein in oilseed crops*. Bunting, E. S. (ed) Martinus Nijhoff Publishers, the Netherlands, 91 - 106 pp.
- Röbellen, G. and Thies, W. (1980a). Biosynthesis of seed oil and Breeding for improved oil quality of Rapeseed. **In:** *Brassica crops and wild allies: Biology and Breeding*. Tsunoda, S., Hinta, K. and Gomez-Campo, C. (eds). Japan Scientific societies press, Tokyo, 253 - 283 pp.

- Röbellen, G. and Thies, W. (1980b). Variation in rapeseed glucosinolates and Breeding for improved meal quality. **In:** *Brassica crops and wild allies: Biology and Breeding*. Tsunoda, S., Hinta, K. and Gomez-Campo, C. (eds). Japan Scientific societies press, Tokyo, 285 - 299 pp.
- Röbellen, G., and Downey, R.K. and Ashri, A. (1989). Oil Crops of the World. Mc Graw-Hill Publishing Co. New York, 447 pp.
- Rosengarten, F. (1969). The Book of spices. Livingston publishing co., wynnemoud, Pennsylvania, 297 - 305 pp.
- Sauer, F.D. and Kramer, J.K.G. (1983). The problems associated with the feeding of high erucic acid rapeseed oils and some fish oils to experimental animals. **In:** *High and low erucic acid rapeseed oils*. Kramer, J. K.G., Sauer, F.G. and Pigden, W.J (eds) Academic Press, New York, 253 - 292 pp.
- Schippers, R.R. (2002). African Indigenous vegetables. An overview of the cultivated species. Natural resources Institute ACP-EU-Technical center for Agricultural and Rural Cooperation. Chatham, UK:
- Seegler, C.J.P. (1983) Oil plants in Ethiopia: Their taxonomy and agricultural significance. Center for agricultural publishing and documentation, PUDOC, Wageningen, the Netherlands.
- Setia, R.C., and Richa. (1989). Anatomical studies on siliquae wall and seed coat development in *Brassica juncea* L Czern and Coss. *Phytomonophology* **39**: 371 - 377.
- Simmonds, N.W. (1979). Longman Principles of crop improvement group, New York.
- Singh, D. (2003). Genetic improvement in Ethiopian mustard (*Brassica carinata* A. Braun) vis a vis Indian mustard (*Brassica juncea* L. Czern and Coss.).In: proc.11th Int. Rapeseed confr. Copenhagen, Denmark, 513pp.
- Tekelewold, A. and Alemayehu, N. (1996). Agro-ecology, distribution and improved production technologies of the highland oil crops in Ethiopia. **In:** Research achievements and technology transfer attempts: Vignettes from shewa: Abera, D. and Beyene, S. (eds) Proc. of the first technology generation, transfer and gap analysis workshop. 25-27 Dec. 1995, Nazret, Ethiopia, 38 - 48pp.

- Teklewold, A. (2005). Diversity study based on quality traits, RAPD Markers, and Investigation of Heterosis in Ethiopian Mustard. PhD.Thesis. George-August University of Göttingen, Germany, 160 pp.
- Tewoldebrihan, G. (1991). Diversity of the Ethiopian flora. **In:** *Plant Genetic Resources of Ethiopia*. Engles, T. M. M. Hanks, T. G and Melaku Worede (eds). University Press, Cambridge 75 - 81 pp.
- Tkachuk, R. (1969). Nitrogen-to-protein conversion factors for cereals and oilseed meals. *Cereal Chem.* **46**: 419 - 442.
- Tsige, G. Labuschagne, M.T. and Hugo, A. (2005b). Genetic relationships among Ethiopian Mustard genotypes on oil content and Fatty acid composition. *Afri. J. of Biotech.* **4**: 1256 - 1268.
- Tsige, G. Viljoen, C., and Labuschagne, M. (2005a). Genetic analysis of Ethiopian mustard genotypes using amplified fragment length polymorphism (AFLP) markers. *Afr. J. Biotech.* **4**: 891 - 897.
- U N (1935). Genome analysis in *Brassica* with species reference to the experimental formation of *B. napus* and peculiar mode of fertilization. *Japanese J. Bot.* **7**: 389 – 452.
- Vaughan, J.G and Hemingway, J.S. (1959). The Utilization of Mustards. *Eco. Bot.* **13**: 196 - 204.
- Velasco, L. and Goffman, D. (2000). Tocopherol, plastochromanol and fatty acid patterns in genus *Linum*. *Plant Syst. Evol.* **221**: 77 - 88.
- Velasco, L., Fernandez-Martinez, J. and DeHaro, A. (1995). The applicability of NIRS for estimating multiple seed quality components in Ethiopian mustard. **In:** Proc. 9th Int. Rapeseed Conf., Cambridge, UK. 867 – 869 pp.
- Velasco, L., Goffman, F.D. and Becker, H.C. (1998). Variability for the fatty acid composition of the seed oil on germplasm collection of the genus *Brassica*. *Genet. Resour.Crop. Evol.* **45**: 371 - 382.
- Velasco, L., Nabloussi, A., Haro, A.D. and Fernandez-martinez, J. (2004). Allelic variation in linoleic acid content of high erucic acid Ethiopian Mustard and

- incorporation of the low linoleinic acid trait into zero erucic acid germplasm. *Plant breed.* **123**: 137 - 143.
- Wesphal, A. and Marquard, R. (1980). Yield quality of *Brassica* species in Ethiopia. *Palnt. res. Dev.* **13**: 1 - 11.
- Yalden, W.D. (1983). The extent of high ground in Ethiopia compared to the rest of Africa. *SINET. Eth. J. sci.* **6**: 35 - 38.
- Yang, Y.W. Tseng, P.F., Tai, T.Y. and Chang, C.J. (1998). Phylogenetic position of *Raphanus* in relation to *Brassica* species based on 5S rRNA spacer sequence data. *Bot. Bull. Acad. Sci.* **39**: 153 - 160.
- Yitbarek, S. (1992). Pathological research on Noug, linseed, Gomenzer and rapeseed in Ethiopia. **In**: Oilseeds research and development in Ethiopia, Proceedings of the First National Oilseeds Workshop, 3 - 5 December 1991, Addis Ababa, Ethiopia 151 - 161 pp.
- Zelleke, A. and Mariam, S.G. (1991). Role of research for horticultural development in Ethiopia. International Symposium on Horticultural Economics in Developing Countries. Alemaya, Ethiopia, 189 - 196 pp.
- Zhao, J. (2002). QTLs for oil content and their relationships to other agronomic traits in an European x Chinese oilseed rape population. PhD. Thesis available at http://Webdoc.sub.gwdg.de/diss/2002/Zhao_jianyi/Zhao_jianyi.pdf. Georg-August University of Göttingen, German.

APPENDICES

Appendix 1 Result of seed quality traits of seeds of *B. carinata* and *B. napus*.

S/N	Accession No.	% Protein Content	Mean	% Fat Content	Mean	% fiber Content	Mean	% Mineral Content	Mean	% Moisture Content	Mean	% Carbohydrate Content	Mean
1	203230	17.0943		47.4301		13.4027		3.2298		3.9384		28.3074	
	203230	16.8466		47.3572		12.4651		3.2043		4.1098		28.4821	
	203230	17.8995	17.2801	47.6822	47.4898	11.7991	12.5556	3.2660	3.2334	4.1737	4.0740	26.9785	27.9227
2	215790	19.3860		47.1348		8.4360		2.3401		4.1498		26.9892	
	215790	17.9614		46.7168		8.4326		2.6592		4.1198		28.5428	
	215790	18.9834	18.7769	47.1115	46.9877	9.1428	8.6704	2.6573	2.5522	4.0444	4.1047	27.2034	27.5785
3	203223	19.4169		46.2825		6.2572		3.3261		4.6186		26.3559	
	203223	18.5808		46.6297		6.5910		3.2990		4.6331		26.8574	
	203223	19.7886	19.2621	46.7025	46.5382	5.6330	6.1604	3.2997	3.3083	4.5393	4.5970	25.6699	26.2944
4	207918	17.7756		46.7881		7.5854		3.4768		4.2342		27.7254	
	207918	16.2582		47.2136		8.2664		3.4303		4.2096		28.8883	
	207918	17.3111	17.1150	47.0139	47.0052	7.2253	7.6923	3.3823	3.4298	4.1248	4.1895	28.1679	28.2605
5	208590	19.5718		46.4247		9.4213		2.9036		4.3939		26.7060	
	208590	18.9524		46.4073		8.8787		2.9113		4.5232		27.2058	
	208590	20.3150	19.6131	46.2034	46.3451	9.9697	9.4232	2.9303	2.9151	4.4584	4.4585	26.0929	26.6683
6	21094	23.4737		41.6419		8.5432		3.3441		4.5143		27.0260	
	21094	21.2440		41.3196		8.2237		3.3080		4.4989		29.6295	
	21094	23.8763	22.8647	41.0807	41.3474	8.4350	8.4006	3.2978	3.3166	4.4934	4.5022	27.2518	27.9691
7	212224	19.9124		49.9056		7.1764		2.7020		4.1685		23.3115	
	212224	19.1692		49.6875		5.5984		2.7245		3.9786		24.4402	
	212224	19.8195	19.6337	49.9738	49.8556	8.1731	6.9826	2.6906	2.7057	3.8786	4.0086	23.6375	23.7964
8	208961	18.3021		47.7731		8.1160		3.2443		3.7339		26.9467	
	208961	18.3331		47.8748		10.2968		3.2485		3.8733		26.6703	
	208961	18.0234	18.2195	47.5306	47.7262	9.2162	9.2097	3.2394	3.2441	3.8044	3.8039	27.4021	27.0064
9	21070	20.9344		43.4266		8.2913		3.7082		4.5541		27.3767	

	21070	20.4079		43.4695		7.5731		3.7026		4.5882		27.8318	
	21070	21.3679	20.9034	43.3074	43.4012	7.9616	7.9420	3.7193	3.7100	4.4837	4.5420	27.1218	27.4434
10	20130	17.6208		49.3448		6.7253		3.5567		3.9636		25.5141	
	20130	17.1872		49.3685		5.7018		3.5353		3.9386		25.9704	
	20130	17.4040	17.4040	49.5019	49.4050	5.7233	6.0501	3.6621	3.5847	3.7937	3.8986	25.6383	25.7076
11	207919	17.8376		47.1930		9.3555		2.8377		4.1683		27.9634	
	207919	16.5679		46.5696		10.0658		2.8418		4.0696		29.9511	
	207919	16.5369	16.9808	47.2416	47.0014	11.1616	10.1943	2.8092	2.8296	4.0234	4.0871	29.3888	29.1011
12	208594	16.7227		49.2620		7.6559		3.4590		4.3339		26.2223	
	208594	16.0414		49.2814		8.2608		3.4002		4.4248		26.8522	
	208594	17.0014	16.5885	48.8541	49.1325	8.8590	8.2586	3.3692	3.4095	4.4389	4.3992	26.3363	26.4703
13	208611	23.2879		44.3542		8.9040		2.5760		4.3982		25.3836	
	208611	21.3679		44.3437		8.6039		2.6623		4.3648		27.2614	
	208611	23.0712	22.5757	44.3549	44.3509	9.0474	8.8518	2.6264	2.6215	4.4598	4.4076	25.4877	26.0442
14	202945	17.8066		44.5634		8.3919		3.6016		4.5982		29.4302	
	202945	16.2272		44.4327		8.6190		3.6449		4.4546		31.2407	
	202945	17.4969	17.1769	44.5064	44.5008	8.0005	8.3371	3.7252	3.6572	4.4480	4.5002	29.8235	30.1648
15	203225	19.5408		47.8825		8.9624		3.5495		4.3335		24.6938	
	203225	17.1872		47.5308		8.0658		3.4386		3.8392		28.0042	
	203225	18.2092	18.3124	47.8212	47.7448	9.6849	8.9044	3.4646	3.4842	3.6191	3.9306	26.8859	26.5279
16	208592	19.5718		45.0800		4.9663		2.7716		4.1737		28.4028	
	208592	19.6647		45.2579		5.3958		2.8324		4.2044		28.0406	
	208592	20.1911	19.8092	45.2476	45.1952	4.3701	4.9108	2.8372	2.8138	4.1498	4.1760	27.5744	28.0059
17	208599	19.5408		47.5229		10.2567		2.7830		4.1987		25.9546	
	208599	19.0144		47.1132		10.8547		2.8303		4.1540		26.8882	
	208599	19.4789	19.3447	47.5410	47.3924	9.2567	10.1227	2.7991	2.8041	4.0286	4.1271	26.1525	26.3317
18	200405	13.6259		52.1863		6.0664		3.1917		3.3145		27.6815	
	200405	14.7408		49.4320		5.6534		3.2003		3.4590		29.1679	
	200405	15.2982	14.5550	49.3254	50.3146	6.5405	6.0867	3.2399	3.2107	3.4191	3.3975	28.7173	28.5223
19	208585	20.3460		32.5909		9.6564		2.8345		4.0588		40.1698	
	208585	19.8195		30.3625		10.6344		2.8491		3.9642		43.0047	
	208585	20.5008	20.2221	31.9054	31.6196	10.7544	10.3484	2.8090	2.8308	4.0134	4.0121	40.7715	41.3153
20	21076	21.0892		41.1447		13.3606		3.7936		4.1592		29.8133	

	21076	20.5628		40.9385		13.2212		3.8982		4.1698		30.4307	
	21076	20.6557	20.7692	40.3840	40.8224	13.5009	13.3609	3.8755	3.8558	3.6939	4.0076	31.3910	30.5450
21	21217	18.3640		45.6920		7.1020		3.8045		4.6934		27.4461	
	21217	16.9085		46.2634		5.8175		3.9316		4.4648		28.4318	
	21217	17.9305	17.7343	44.7075	45.5543	6.7404	6.5533	3.6522	3.7961	3.1792	4.1124	30.5306	28.8028
22	Yellow-D.	18.0853		49.6458		5.6684		3.3070		4.2541		24.7077	
	Yellow-D.	16.5988		49.5561		6.8593		3.3125		3.8190		26.7135	
	Yellow-D.	18.3331	17.6724	49.5175	49.5731	10.3782	7.6353	3.2816	3.3004	4.0294	4.0342	24.8384	25.4199
23	Holleta-1	18.5808		44.8982		6.7297		4.0095		4.3244		28.1871	
	Holleta-1	18.1782		45.5358		8.5875		3.9703		4.5693		27.7465	
	Holleta-1	18.9524	18.5705	45.4709	45.3016	8.7733	8.0302	3.9427	3.9742	4.5598	4.4845	27.0742	27.6692
24	S-67	17.9924		47.6205		8.9738		3.9791		4.9193		25.4888	
	S-67	18.0853		47.4517		8.6763		3.9626		4.6341		25.8663	
	S-67	18.0234	18.0337	46.0151	47.0291	9.8224	9.1575	3.9357	3.9591	4.6436	4.7323	27.3822	26.2458
25	Pura	22.9473		48.0671		9.7692		3.4841		4.1442		21.3574	
	Pura	18.3021		48.0689		8.0699		3.5056		4.1648		25.9587	
	Pura	19.4789	20.2428	47.8703	48.0021	8.2763	8.7051	3.5145	3.5014	4.3237	4.2109	24.8126	24.0429
26	Tower-S-3	16.0724		50.2046		9.4162		3.3737		4.2839		26.0654	
	Tower-S-3	15.3911		50.0568		9.5732		3.3868		4.1646		27.0007	
	Tower-S-3	14.9561	15.4732	49.9351	50.0655	9.0481	9.3458	3.3673	3.3759	4.2987	4.2491	27.4427	26.8363
27	Tower	18.6427		48.7782		10.2449		3.4375		5.0832		24.0583	
	Tower	18.7356		48.4992		10.0790		3.4735		4.9983		24.2934	
	Tower	18.9834	18.7872	48.3967	48.5580	10.0148	10.1129	3.7783	3.5631	5.0882	5.0566	23.7534	24.0350

Appendix 2 Significant tests of variation of seed quality traits between the areas.

Protein content

	Sum of Squares	df	Mean Square	F	Sig.
Between areas	42.006	2	21.003	5.630	.006
Within areas	223.838	18	3.731		
Total	265.845	20			

Post Hoc Tests

Multiple Comparisons

(I) Region for protein collection	(J) Region for protein collection	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Shewa	Wellga	.4350	.5961	1.000	-1.0331	1.9031
	Eastern Oromia	-1.4732*	.5961	.049	-2.9413	-5.1134E-03
Wellega	Shewa	-.4350	.5961	1.000	-1.9031	1.0331
	Eastern Oromia	-1.9082*	.5961	.007	-3.3763	-.4401
Eastern Oromia	Shewa	1.4732*	.5961	.049	5.113E-03	2.9413
	Wellega	1.9082*	.5961	.007	.4401	3.3763

* The mean difference is significant at the .05 level

Appendix 2 continued

Moisture content

	Sum of Squares	df	Mean Square	F	Sig.
Between areas	.796	2	.398	3.828	.027
Within areas	6.237	18	.104		
total	7.033	20			

Post Hoc Tests

Multiple Comparisons

(I) Regions for collecting Moisture content	(J) Regions for collecting Moisture content	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence interval	
					Lower Bound	Upper Bound
Shewa	Wellega	.1177	9.950E-02	.725	-.1274	.3627
	Eastern Oromia	-.1567	9.950E-02	.362	-.4018	8.837E-02
Wellega	Shewa	-.1177	9.950E-02	.725	-.3627	.1274
	Eastern Oromia	-.2744*	9.950E-02	.023	-.5194	-2.9314E-02
Eastern Oromia	Shewa	.1567	9.950E-02	.362	-8.8371E-02	.4018
	Wellega	.2744*	9.950E-02	.023	2.931E-02	.5194

* The mean difference is significant at the .05 level.

Appendix 3 Significance test of variation between the entire seed sample for the six proximate compositions.

Proximate composition		Sum of squares	df	Mean square	F	Sig.
Protein	Between samples	288.630	26	11.101	16.966	.000
	Within samples	35.333	54	.654		
	Total	323.963	80			
Fat	Between samples	1128.686	26	43.411	185.098	.000
	Within samples	12.665	54	.235		
	Total	1141.351	80			
Fibre	Between Groups	277.588	26	10.676	16.464	.000
	Within Groups	35.017	54	.648		
	Total	312.605	80			
Mineral	Between samples	13.377	26	.515	118.340	.000
	Within samples	.235	54	.004		
	Total	13.612	80			
Moisture	Between samples	8.520	26	.328	8.156	.000
	Within samples	2.169	54	.040		
	Total	10.689	80			
Carbohydrate	Between samples	806.206	26	31.008	31.740	.000
	Within samples	52.754	54	.977		
	Total	858.961	80			

Appendix 4 Descriptive statistics of the samples from the three altitude classes.

Altitude classes		Protein	Fat	Fiber	Mineral	Moisture	Carbohydrate
Class 1	Mean	17.949389	44.267444	9.042333	3.178667	4.274456	30.330044
	N	18	18	18	18	18	18
	Std. Deviation	1.5611823	6.0003219	1.1578582	.3422568	.2008714	5.2821701
	Minimum	16.0414	30.3625	7.2253	2.8090	7.9642	26.0929
	Maximum	20.5008	49.2814	11.1616	3.7252	4.5982	43.0047
	Range	4.4594	18.9189	3.9363	.9162	.6340	16.9118
	Std. Error of Mean	.3679742	1.4142894	.0806707	.0806707	.0473458	1.2450194
Class 2	Mean	20.189411	45.726911	3.014506	3.014506	4.278194	26.790989
	N	18	18	18	18	18	18
	Std. Deviation	2.0415743	2.3143963	.3844477	.3844477	.2792972	1.1869346
	Minimum	17.1872	41.0807	2.3401	2.3401	3.6191	24.6938
	Maximum	23.8763	47.8825	3.5495	3.5495	4.6331	29.6295
	Range	6.6891	6.8018	1.2094	1.2094	1.0140	4.9357
	Std. Error of Mean	.4812037	.5455061	.0906152	.0906152	0.658310	.2797532
Class 3	Mean	18.319289	46.968444	3.244928	3.244928	4.051750	27.415583
	N	18	18	18	18	18	18
	Std. Deviation	2.2074511	2.6530623	.4228860	.4228860	.4533120	1.8679720
	Minimum	13.6259	43.3074	2.6906	2.6906	3.1792	23.3115
	Maximum	21.3679	52.1863	3.9316	3.9316	4.6934	30.5306
	Range	7.7420	8.8789	1.2410	1.241-	1.5142	7.2191
	Std. Error of Mean	.5203012	.6253330	.0996752	.0996752	.1068467	.4402852
Total	Mean	18.819363	45.654267	8.355300	3.146033	4.201467	28.178872
	N	54	54	54	54	54	54
	Std. Deviation	2.1589653	4.0945982	1.8795820	.3897443	0.3395543	3.5978122
	Minimum	13.6259	30.3625	4.3701	2.3401	3.1792	23.3115
	Maximum	23.8763	52.1863	13.4027	3.9316	4.6934	43.0047
	Range	10.2504	21.8238	9.0326	1.5915	1.5142	19.6932
	Std. Error of Mean	.2937980	.5572042	.2557787	.0530375	.462075	0.4896002

Appendix 5 significance test of the between altitude classes comparison of the six proximate compositions.

Proximate composition		Sum of Squares	df	Mean Square	F	Sig.
Protein	Between altitude classes	51.911	2	25.956	6.784	.002
	Within altitude classes	195.129	18	3.826		
	Total	247.040	20			
Fat	Between altitude classes	65.801	2	32.901	2.039	.141
	Within altitude classes	822.783	18	16.133		
	Total	888.584	20			
Fiber	Between altitude classes	21.984	2	10.992	3.392	.041
	Within altitude classes	165.256	18	3.240		
	Total	187.240	20			
Mineral	Between altitude classes	.507	2	.253	1.712	.191
	Within altitude classes	7.544	18	.148		
	Total	8.051	20			
Moisture	Between altitude classes	.605	2	.303	2.804	.070
	Within altitude classes	5.505	18	.108		
	Total	6.111	20			
Carbohydrate	Between altitude classes	128.455	2	64.227	5.875	.005
	Within altitude classes	557.591	18	10.933		
	Total	686.045	20			