1 General Introduction

1.1 *Brassica carinata*: an overview

Ethiopian mustard (*Brassica carinata* A. Braun), locally known as "Gomenzer", is among the oldest crops cultivated in Ethiopia. Its cultivation is believed to date back in the 4th to 5th Millennium BC (Simmonds 1979). *B. carinata* is an amphidiploid species (BBCC, n = 17) containing the BB genome of *B. nigra* (n = 8) and CC genome of *B. oleracea* (n = 9) (U 1935). The crop is believed to evolve in the highlands of Ethiopia and adjoining portion of East Africa and the Mediterranean coast (Simmonds 1979; Hemingway 1995; Gomez-Campo and Prakash 1999).

In Ethiopia, *B. carinata* is grown as oilseed and vegetable crop. At its earlier stages of development the leaf is used as vegetable either by thinning or topping and seed can be also harvested from the same plant for oil extraction and other uses that include: greasing traditional bread-baking clay pan (oven), curing certain ailments and preparing beverages (Alemayehu 2001). Using the same plant as sources of vegetable and seed might have retarded the differentiation of the crop to distinct vegetable and oilseed types.

Investigations on non-traditional use of the crop witnessed that, after trans-esterification, the oil exhibit physical and chemical properties suitable for bio-diesel (Cardone et al. 2003). The crop has a potential to be used as feedstock for oleochemichals (due to high erucic and linolenic acids) and bio-fumigant (due to its high glucosinolate) industries.

B. carinata is grown in most parts of Ethiopia situated above 1700 m a.s.l. (Teklewold and Alemayehu 1996) by smallholder farmers in more fertile and well-drained fields, usually around homesteads. According to the agriculture sample survey of 2002/2003 (CSA 2003), the national average productivity of *B. carinata* is the highest (about 1.13 tonnes hectare^{-1}) but is grown only on 17, 256 hectares, the third smallest area cropped by the six species of oilseeds grown in the country. Production trend analysis in Ethiopia shows that the area and production of *B. carinata* has increased between 1982 and 2003 by 575% and 1044%, respectively (CSA 1987, 2003). Farmers' attraction to the crop could still be enhanced through the release of high yielding varieties with better quality.

B. carinata has become an object of interest in Canada (Rakow 1995), Spain (Velasco et al. 1995a), India (Singh 2003), America and Italy (Cardone et al. 2003) due to its draught tolerance superiority over the other oilseed *Brassicas. B. carinata* possesses deep root, low

canopy temperature, thick waxy leaves and high temperature tolerance characters that suited the crop to moisture stressed conditions (Singh 2003). It is reputed also as insect pest (Tadesse and Bayeh 1992), disease (Gugel et al. 1990; Singh 2003) and shattering (Alemayehu and Becker 2002) resistant. Wide availability of yellow seeded genotypes that yield low fibre with high oil and protein contents (Getinet et al. 1996a) and its role in crop rotation as a break-crop in cereals-based mono cropping are other attributes of the crop. In recent years, its quality attributes has been transferred through inter-specific hybridisation to improve the other *Brassica* species (Meng et al. 1998; Singh 2003).

As a field crop, the cultivation of *B. carinata* in the world has hitherto been localized to Ethiopia only. This may be intrinsically associated to its low oil and meal qualities. Even in its native place, Ethiopia, consumers have the least preferences to its oil, so does the cattle for the meal. The oil from the seed is characterized by a high level of erucic acid (> 40%) and excessive amount of linolenic acid (Becker et al. 1999). Feeding experiments in rats showed an increased level of erucic acid to be associated with reduced food intake, poor digestibility, weight losses, myocardial lipidosis and death (Sauer and Kramer 1983). Because of its three double bonds, linolenic acid is sensitive to autoxidation resulting in flavour reversion and short-chain degradation products, some of which are even claimed to be metabolically harmful (Röbbelen and Thies 1980a). The meal obtained after oil extraction, although it contains about 39% crude protein (Seyoum 1995), is not preferred for feeding due to the presence of an excessive amount of glucosinolates (Röbbelen and Thies 1980b; Becker et al. 1999). Glucosinolates are diverse group of sulphur-containing glycosides present in all cruciferous plants which upon hydrolysis released a variety of products showing toxic and antinutritive effects, limiting the potential uses of the meal (Bones and Rossiter 1996). Intact glucosinolate have generally been considered to be innocuous. Hydrolysis products, on the other hand, produce several physiological effects when they are present in large quantities in the animal feeds. These include: depressed growth related to the goiterogencity of several hydrolysis products, haemorrhagic livers in poultry possibly related to the presence of epithionitriles, and skeletal abnormalities in poultry (Röbbelen and Thies 1980b; Downey 1990; Bell 1993; Uppstrom 1995; Becker et al. 1999). For human use, glucosinolate are important as flavour compounds (in vegetable and condiment species), cancer-preventive agents, and biopesticides (Fenwick et al. 1983; Uppstrom, 1995; Mikkelsen et al. 2000).

Research experiences in Canada and many countries of Western Europe suggested that consumers preference to the other oilseed *Brassicas* (*B. napus*, *B. rapa* and *B. juncea*) have been greatly enhanced by altering their fatty acid profile such that linolenic and erucic acids from the oil and glucosinolates from the meal get reduced (Röbbelen and Thies 1980a; Becker et al. 1999). Germplasm of *B. carinata* with low erucic acid levels were released (Fernandez-Martinez et al. 2001) but the lack of low glucosinolate containing genotypes is still a hindrance to develop the crop as nutritionally acceptable oilseed *Brassica*.

1.2 Assessment of genetic diversity

Genetic diversity measures individual variation and reflects the frequency of different types in a population (Frankel et al. 1995). Genetic variation can be assembled and utilized through hybridisation to create new favourable gene combination and crosses between genetically divergent parents are expected to result with large genetic variance among progenies in subsequent selfing generation that can be exploited as line cultivar than crosses from closely related parents. In heterosis breeding, analysis of genetic diversity helps to select parents or tester for maximizing heterotic response. Similarly, determining the genetic variation among and within germplasm accessions facilitates reliable classification of accessions and identification of subset core accessions with possible utility for specific breeding purpose and to preserve maximum genetic diversity in germplasm sources (Thormann and Osborn 1992; Mohammadi and Prasanna 2003). Thus, detecting and quantifying the degree of dissimilarity among species, subspecies, populations, and elite breeding material is of primary concern in population genetics and plant breeding (Rief et al. 2005).

Genetic architecture of a population is generally believed to be the result of breeding system, gene flow within and between population, isolation mechanism and prolonged selection by various natural and artificial forces (Chandel and Joshi 1983). The genetic variation within a taxon is not uniformly distributed through out the geographic area where it is growing (Frankel et al. 1995) and populations from area far separated are normally expected to accumulate enormous genetic diversity (Chandel and Joshi 1983; Singh 1996). In cultivated crop species, geographical distribution patterns reflect both the specific selection pressures prevailing in a particular environment as well as history of selection and production (Hawtin et al. 1997). According to Linhart and Grant (1996) and Rao and Hodgkin (2002), natural selection acting on heritable phenotypic variation will result in adaptation and

differentiation among populations of the same species inhabiting environments differing in their selective regime. In a diversity study, therefore, the inclusion of genotypes collected from different geographic areas has been adopted as a strategy to capture most of the allelic diversity of a particular species.

Studies conducted to determine the extent of genetic diversity in *B. carinata* are limited. In characterizing accessions of *B. carinata* collected from different parts of Ethiopia, Abebe et al. (1992) observed the presence of wide variation for morphological and agronomic traits. Alemayehu and Becker (2002), by studying 36 genotypes of Ethiopian origin, showed the presence of large genetic diversity for agronomically important traits. They noted no definite correspondence between genetic and geographic diversities but some traits do occur more often in a certain geographical region than the others.

Established methods for characterizing and measuring genetic diversity have relied on morphological, physiological and cytological markers as well as biometric analyses of quantitative and qualitative traits, heterosis, or the segregation variance in crosses (Melchinger 1999). Except the cytological markers, the others are used for assessing quantitative differences, which are controlled by multiple genes and are liable to environmental modifications; hence, genetic differences could not be absolute (Liu and Furnier 1993). To alleviate this problem, biochemical and molecular approaches such as iso- and allozymes, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), microsattelite, amplified fragment length polymorphism (AFLP), single nucleotide polymorphism (SNP), etc have been employed to assess genetic relationships. Molecular markers bring new information on domestication of agricultural plants, their diffusion and the organisation of genetic diversity within plant species of agricultural interest (Charcosset and Moreau 2004).

Amplification of genomic DNA using at least one short oligonucleotid primer under a set of conditions results in multiple amplification products from loci distributed in the genome. This technique, which does not require any sequence information, was called randomly amplified polymorphic DNA (RAPD) by Williams et al. (1990) and arbitrary primed PCR (AP-PCR) analysis by Welsh and McClelland (1990). RAPD analysis enables the detection of informative genetic markers at a large number of loci in both coding and non-coding regions of the genome (Williams et al. 1990). The method has been applied in the development of genome mapping and finger printing (Williams et al. 1990; Welsh and

McClelland 1990). Random amplified polymorphic DNA markers have been used to estimate genetic distances between *B. oleracea* accessions (Hu and Quiros 1991; Kresovich et al. 1992; Phippen 1997). In the other *Brassica* crops, RAPD markers are considered to be as efficient as RFLP markers for estimating intra-specific genetic relationships among genotypes (Demeke et al. 1992; Thormann et al. 1994; Dos Santos et al. 1994). In the different *Brassica* crops RAPD markers have also been used for cultivar identification (Delourme et al. 1998) and for genetic mapping (Cheung et al. 1997b).

1.3 Heterosis and hybrid breeding

Exploitation of heterosis has played a major role in the genetic improvement of many crop plants and animals (Falconer and Mackay 1996; Stuber 1994) and is considered essential to meet the world's food needs (Duvick 1999; Phillips 1999). Heterosis for yield is larger in cross-fertilized plants like maize reaching about 200% over the mid-parents (Melchinger and Gumber 1998). In the past few decades heterosis has been devised as a competent breeding methodologies to increase yield of the major oilseed crops i.e. soybean, rapeseed, and sunflower (Becker et al. 1999; Miller 1999; Frauen et al. 2003).

Different researchers reported a substantial amount of heterosis in the major oilseed *Brassicas* (for review see Leon and Becker 1995; McVetty 1995). Pradhan et al. (1993) reported a 29 to 92% heterosis over the best yielding parents in *B. juncea* by crossing parents of Indian and exotic origin. Similarly, Banga and Labana (1984) observed a very high level of high-parent heterosis, as high as 263%, in *B. juncea*. Brandle and McVetty (1989) reported high-parent heterosis reaching 120% for seed yield in *B. napus.* Similarly, in *B. napus* Riaz et al. (2001) reported mid-parent heterosis and high-parent heterosis that ranged from 26 to 169% and -4 to 153%, respectively. They also found a positive high-parent heterosis for oil content. In *B. rapa* hybrid, high-parent heterosis for seed yield reaching 60% was reported by Schuler et al. (1992) and Falk et al. (1994).

As appealing the yielding and other advantage of hybrid varieties are, the expansion of *B. napus* hybrid cultivation has been vigorous. In China, only a single hybrid cultivar has occupied about 9.8 million ha for ten years (Dianrong 1999) and the share of hybrids in the total *B. napus* production was projected to reach 40-50% in the year 2000 (Fu and Yang 1995). In 2002/2003, 50% of the 1.27 million ha *B. napus* producing area in Germany was planted by hybrids (Frauen et al. 2003). Mixed seed production (without strip or block

planting) which has been used in USA and Canada reduces seed production cost (Becker et al. 1999; Frauen et al. 2003) and improve its adoption by farmers due to low seed economics. Moreover, the low seed rate in oil seed *Brassica* crops could offset the high cost of hybrid seeds.

In the absence of effective pollination control for hybrid seed production, heterosis could be exploited in partially allogamous crops like *B. carinata* by developing synthetic cultivars (Becker et al. 1999). Synthetic varieties are a special kind of population varieties that are produced by parents that combine well with each other and are maintained by continuous open pollination (Becker 1988; Singh 1996). When a strict uniformity of product is not a requisite, seed of synthetics are simpler to produce than hybrids and can be multiplied by smallholder farmers (Becker et al. 1998) but may not utilize all the available heterosis. The heterogeneous and heterozygous genetic constitution of synthetics contributes to their yield stability over environments (Becker et al. 1998).

Identifying parental combination with increased yield is the most important step in the practical exploitation of the heterosis phenomena (Hallauer and Miranda 1981; Melchinger 1999) but remained part of the art of plant breeding (Smith et al. 1999). Theoretically, the expression of heterosis depends on the difference in the gene frequency and dominance effect of the parental genotypes (Falconer and Mackay 1996). Accordingly, the most productive hybrids may come from parents with higher genetic divergence (Cress 1966; Falconer and Mackay 1996; Melchinger and Gumber 1998). Moreover, genetic diversity of parents is necessary to derive transgressive segregants from a cross. The genetic variance and hence the probability of producing transgressive segregants, increases in proportion to the number of loci for which parents carry different alleles that showed larger genetic variances (Esbroeck and Bowman 1998). In the practical application of hybrid breeding, however, a strong correlation between heterosis and genetic distance is not happening always (Melchinger 1999). But the lack of heterotic response can not warrant the absence of genetic diversity since factors other than diversity are involved in expression of heterosis (Cress 1966). Consequently, research results carried out to determine the relationship between genetic distance and heterosis varied widely from one report to the other based on the study materials and method of genetic distance estimation.

The association of molecular marker and phenotype data based genetic distances with heterosis has been investigated in *B. napus* and *B. juncea*. In accordance to the classical

theories of heterosis, Ali et al. (1995) using morphological markers, Knaak and Ecke (1995) and Diers et al. (1996) using RFLP markers and Riaz et al. (2001) using sequence-related amplified polymorphism (SRAP) observed increase in heterosis with increase in parental divergence in *B. napus*. Falk et al. (1994) in cultivar crosses of *B. rapa* observed that the most heterotic hybrids were produced from crosses between genetically diverse cultivars. However, in resynthesized *B. napus*, Girke et al. (2002) observed low predictive power of RFLP based distance to heterosis. Shen et al. (2003) in *B. napus* found a positive association between hybrid seed yield and genetic distances estimated from AFLP but the coefficient of determination was low. In *B. juncea*, even though genetic distance is useful to select parents with potentially heterotic hybrid combinations, the absence of direct association of genetic distance with heterosis has been reported by Jain et al. (1994) and Sekhon and Gupta (1995).

Relationship between morphological/phenotypic distance and heterosis has also been determined in both *B. napus* and *B. juncea* (Lefort-Buson 1986; Pradhan et al. 1993; Ali et al. 1995). Outcomes from these studies showed the presence of association between parental divergence and either heterosis or F_1 performance, hence indicated a substantial heterosis predicting ability of agronomic traits based distance estimates.

Geographic diversity or origin has been used as a measure of distances to evaluate distance-heterosis association (Moll et al. 1965). In *B. napus* (winter and spring type) the usefulness of geographic origin in assigning heterotic pattern was pointed out by Grant and Beversdorf (1985), Lefort-Buson et al. (1987), Brandle and McVetty (1990). They all reported that crosses between Canadian × European or Asian × European or Europe × Australia or European × Australian genotypes resulted in hybrids with generally higher yield than crosses derived from parents from the same geographic origin. Similarly, Pradhan et al. (1993) in *B. juncea* observed significant better-parent heterosis for single plant yield in Indian × exotic than Indian × Indian or exotic × exotic cross combinations.

B. carinata is the least tailored *Brassica* oil seed species by modern breeding technique. In Ethiopia or elsewhere, breeders still practice line breeding and some forms of population improvement e.g. mass selection for varietal development. Nevertheless, success stories of exploiting hybrid vigour in self-fertilized crops like rice and wheat and partially cross-pollinated crops like *B. napus* justify hybrid and synthetic variety development in *B. carinata* and a corresponding genetic improvement could be visualized by methods akin to those used

by the other oilseed *Brassicas* breeders. But, basic genetic information of heterosis and its utilization has not been previously studied.

Based on the above theoretical premises, experiences on the other *Brassica* oil crops and information gap analysis on the crop, this study was proposed with the following objectives:

- To determine the level of variability for seven seed quality traits (glucosinolate, oil, protein and oleic, linoleic, linolenic and erucic acids) in a segregating progenies;
- To study genetic diversity in relation to geographic origin using RAPD markers;
- To determine the level of heterosis and combining abilities for yield and its components in inbred- and population-derived F_1s;
- To identify pre-harvest plant characteristics and yield components related to observed heterosis for seed yield;
- To estimate some genetic parameters of seed yield and its components;
- To empirically test the predictive value of parental divergence (as determined by geographical origin, molecular markers and agronomic traits) to heterosis; and
- To suggest breeding strategy for utilizing heterosis in *B. carinata*.

2 Variation and Covariation of Seed Quality Traits in Ethiopian Mustard

2.1 Abstract

Ethiopian mustard (*Brassica carinata* A. Braun), an oilseed crop of Ethiopian origin, is less preferred source of edible oil due to its high erucic acid and glucosinolate contents. This study was undertaken to assess the variability for seed quality traits in the Ethiopian germplasm, determine their interrelationships and explore their pattern of genetic variation with respect to geographic origin. Seed of 913 selfed S_2 plants derived from 36 germplasm accessions collected from eight different geographic regions in Ethiopia were analysed for seven seed quality traits by Near Infrared Reflectance Spectroscopy (NIRS). Large variability for oil, protein and glucosinolate contents was observed ranging from 16.4 to 54.7%, 19.0 to 39.3% and 28.2 to 171.8 µmoles g^{-1}, respectively. Fatty acids varied as follow: Oleic, 0 to 20.3%; linoleic, 5.4 to 29.5%; linolenic, 10.1 to 22.9%; and erucic acid 14.3 to 53.3%. Correlation analysis showed a strong negative associations between oil and protein contents and oleic and erucic acids. Mahalanobis` dissimilarity index showed high diversity and the 913 S_2 plants were grouped by the unweighted pair-group method using arithmetic mean (UPGMA) into 11 clusters, that varied in constellation from a cluster containing a single plant to a cluster containing 332 plants. Factors other than geographic origin appeared to be a potent source of genetic diversity and quality improvement may not be expected to have negative effect on adaptation of genotypes to specific environmental conditions. The result demonstrated the usefulness of assessing variation in segregating progenies to detect rare but desired genetic variations like low glucosinolate content.

2.2 Introduction

The value and usefulness of vegetable oil is determined by its fatty acid composition and other seed oil quality parameters (McVetty and Scarth 2002). Despite its long history of cultivation in Ethiopia (Simmonds 1979), the use of Ethiopian mustard (*Brassica carinata* A. Braun) as source of edible oil has been limited because the oil is characterized by high level of erucic (> 40%) and linolenic acids (Becker et al. 1999). The meal obtained after oil extraction contains about 39% crude protein (Seyoum 1995). However, it is not preferred as feed for animals due to the presence of high amount of glucosinolate. In Canada and many