MORPHO-PHYSIOLOGICAL, SEED OIL COMPOSITIONAL, AND MOLECULAR DIVERSITY IN ETHIOPIAN MUSTARD (*Brassica carinata* A. Braun) GERMPLASM

PhD DISSERTATION

BY

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HAWASSA UNIVERSITY, HAWASSA, ETHIOPIA

APRIL 2025

MORPHO-PHYSIOLOGICAL, SEED OIL COMPOSITIONAL, AND MOLECULAR DIVERSITY IN ETHIOPIAN MUSTARD (*Brassica carinata* A. Braun) GERMPLASM

DISSERTATION

SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES

HAWASSA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE AWARD OF THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

PLANT BIOTECHNOLOGY

BY

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This is to certify that the PhD dissertation entitled "**Morpho-Physiological, Seed Oil Compositional, and Molecular Diversity in Ethiopian Mustard (*Brassica carinata* A. Braun) Germplasm"** submitted in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Plant Biotechnology** to the Graduate Program of the School of Plant and Horticultural Sciences, College of Agriculture, and is a record of original research carried out by **Yirssaw Demeke Ambaw**, (**ID No. PhD/Pt.Biotech/009/10**) under our supervision and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been well acknowledged. Therefore, we recommend that it be submitted as fulfilling the requirements of the dissertation.

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STATEMENT OF AUTHOR

I hereby declare that this dissertation is my original work and all sources of materials used for its preparation have been duly acknowledged. This dissertation has been submitted in partial fulfillment of the requirements for the award of the degree of Doctor of Philosophy in Plant Biotechnology at the Hawassa University and is deposited at the University Library to be made available to users under the rules and regulations of the library. I solemnly declare that this dissertation has not been submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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DEDICATION

I dedicate this dissertation to my wife, Kidist Mokyalew, and my children, Maya, Hymn, Yemaryam and Yostina, whose love and unwavering support have been my foundation throughout this academic journey.

BIOGRAPHICAL SKETCH

The author, Yirssaw Demeke Ambaw, was born on September 17, 1987, in Woldya, Ethiopia. He completed his primary education in Addis Alem, Gedober and Woldya and senior secondary and preparatory education at Bole Gerji, Lem, and Dejiazmach Wondyirad schools in Addis Ababa from 1995 to 2003. In 2004, he enrolled at the Jimma University College of Agriculture and Veterinary Medicine and obtained his Bachelor of Science degree in Horticulture in 2007. After graduation, he worked as a production manager at the Ethio-Agri-Ceft Floriculture Company, a MEDROC investment group in Holeta, Ethiopia. He then joined the Department of Horticulture, Samara University, Samara, Ethiopia, and served as a founding faculty member for a year. In 2008, he joined the School of Graduate Studies at Haramaya University and completed his Master of Science degree in Horticulture in 2010. He subsequently worked in lecturer positions at Samara, Ambo, and Jimma Universities, where he was actively engaged in teaching and research. Since 2013, he has been a faculty member at the School of Plant and Horticultural Sciences, Hawassa University, continuing his contributions to both teaching and research. He also attended short professional development training sessions at prestigious institutions, such as the Hebrew University of Jerusalem, the Volcani Research Center in Israel, the Czech University of Life Sciences Prague, and the Norwegian University of Life Sciences. He is the author of over seven peer-reviewed international journal articles and co-authored student instructional books on Fruit Production and Postharvest Handling of Horticultural crops. He began his Doctor of Philosophy in Plant Biotechnology program, a joint program at Hawassa University and the Norwegian University of Life Sciences in 2019, and completed it in 2024. He is also a married father.

ACKNOWLEDGMENTS

Utmost praise and gratitude to the divine Almighty for granting me good health and determination, which supported me during my academic journey.

I am grateful to my advisors, Dr. Andargachew Gedebo and Dr. Temesgen Magule, for their invaluable guidance and support throughout my research and thesis writing. Their expertise and insightful suggestions were instrumental in the completion of this study.

I appreciate my employer, Hawassa University for providing study leave and financial assistance. Special thanks to the Norwegian University of Life Sciences (NMBU) - Hawassa University (HU) Institutional Collaboration Phase-IV (NMBU_HU ICP_IV) project for their financial support and facilitation of the exchange program at NMBU, Norway. I also extend my gratitude to the School of Plant and Horticultural Sciences and the College of Agriculture at Hawassa University for their support.

I sincerely thank the Ethiopian Biodiversity Institute (EBI) for providing Ethiopian mustard germplasm materials. I am immensely grateful to the Holeta Agricultural Research Center (HARC) for supplying additional material and facilitating the research field and laboratory facilities. Additionally, I appreciate the Agriculture Offices in Hulla District, Sidama Region, and Basona Worena District, Amhara Region, for their assistance with landrace collection.

I heartly thank Mr. Birhanu Mengistu and the high- and mid-land oilseed improvement program team at the HARC for their assistance with research field management and data collection. Special thanks to Prof. Tesfaye Abebe, Dr. Hewan Demisse, Dr. Mikias Biyazen, Mulugeta Adamu, Belay Tolera for their invaluable contributions. I also acknowledge the SPLHO research field and laboratory assistants: Genet Buche, Tsehay

Kora, Yeshewaharge Zeleke, and Behabtua Degu for their contribution in seedling raising and leaf sample preparation. I am thankful to all my colleagues and friends for their insightful comments, encouragement, and solidarity during the successful completion of my research.

I am deeply grateful to my devoted parents, Ato Demeke Ambaw and W/ro Kebebush Akele, and my cherished sisters, Yerom Demeke and Aseged Demeke, for their unwavering support, unconditional love, and prayers that have significantly contributed to my accomplishments.

Special thanks are due to my exceptional wife Kidist Mokyalew, whose enduring encouragement and steadfast presence have been pivotal in my academic endeavors. Her love, support, prayers, and dedication to our children have been indispensable to the successful completion of this research. I also extend my appreciation to my adorable daughters, Maya Yirssaw, Hymn Yirssaw, Yemaryam Yirssaw, and Yostina Yirssaw whose presence has brought immense joy, inspiration, and solace throughout the long journey of pursuing a PhD.

Ultimately, I am indebted to all those who have contributed to this work.

LIST OF ACRONYMS AND ABBREVIATIONS

ABD	Augmented Block Design
Acc	Accession
AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of molecular variance
ANOVA	Analysis of Variance
CV	Coefficient of Variation
DArT	Diversity Arrey Technology
EBI	Ethiopian biodiversity Institute
EIAR	Ethiopian Institute of Agricultural Research
FAOSTAT	Food & Agriculture Organization of the United Nation Statstics
FIS	Inbreeding Coefficient
FST	Fixation Index
GA	Genetic Advance
GAM	Genetic Advance of Mean as Percentage
GBS	Genotyping by Sequencing
GCV	Genotypic Coefficient of Variance
gDNA	Genomic DNA
GS	Genomic Selection
GWAS	Genome-Wide Association Studies
H^2	Heritability in Broad Sense
HARC	Holeta Agricultural Research Center
IBPGR	International Board for Plant Genetic Resources
K	Selection Intensity
MAF	Minor Allele Frequency

MaF	Major Allele Frequency
MAS	Marker-Assisted Selection
MCMC	Markov Chain Monte Carlo
MoA	Ministry of Agriculture
MPCA	Multivariate principal component analysis
MUFAs	Mono Unsaturated Fatty Acids
NIRS	Near Infrared Reflectance Spectroscopy
Nm	Number of migrants
NMRS	Nuclear Magnetic Resonance Spectroscopy
PC	Principal Component
PCA	Principal Component Analysis
PCoA	Principal Coordinate Analysis
PCV	Phenotypic Coefficient of variation
PhiPT	Genetic Differentiation
PIC	Polymorphic Information Content
PUFAs	Poly Unsaturated Fatty Acids
R^2	Coefficient of Determination
RAPD	Random Amplification Polymorphic DNA
SAS	Statistical Analysis Software
SE	Standared Error
SEQART	Sequential Art
SNPs	Single Nucleotide Polymorphisms
SSR	Simple Sequence Repeat
UPGMA	Unweighted Pair Group Method with Arithmetic mean

PAPERS/MANUSCRIPT TITLES

Chapter II: Analyzing the Diversity within Ethiopian Mustard (*Brassica carinata* A. Braun) Germplasm for Quantitative Agro-Morphological and Physiological Traits

Published as: Yirssaw Demeke Ambaw, Andargachew Gedebo Abitea, Temesgen Magule Olango and Birhanu Mengistu Aboye. 2024. Agromorphological and Physiological Trait Diversity in Ethiopian Mustard (*Brassica carinata* A. Braun) Germplasm. Advances in Agriculture. Volume 2024, Article ID: 2398294. https://doi.org/10.1155/aia/2398294

Chapter III: Evaluating the Variability in Seed Oil Content and Fatty Acid Composition of Ethiopian Mustard (*Brassica carinata* A. Braun) Germplasm

Published as: Yirssaw Demeke Ambaw, Andargachew Gedebo Abitea, Temesgen Magule Olango and Mikias Biazen Molla. 2024. Genetic Variation in Ethiopian Mustard (*Brassica carinata* A. Braun) Germplasm based on Seed Oil Content and Fatty Acid Composition. Genetic Resources and Crop Evolution. Article no. 2155. Doi: https://doi.org/10.1007/s10722-024-02155-4

Chapter IV: Estimating Genetic Diversity and Population Structure in Ethiopian Mustard (*Brassica carinata* A. Braun) Germplasm using DArTseq-generated SNP Markers

Published as: Yirssaw Demeke Ambaw, Andargachew Gedebo Abitea and Temesgen Magule Olango. 2025. Genetic Diversity and Population Structure in Ethiopian Mustard (*Brassica carinata* A. Braun) Revealed by High-Density DArTSeq SNP Genotyping. BMC Genomics 26, 354 (2025). https://doi.org/10.1186/s12864-025-11469-1

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Thesis Abstract

Morpho-Physiological, Seed Oil Compositional and Molecular Diversity in Ethiopian Mustard (*Brassica carinata* A.Braun) Germplasm

Ethiopian mustard (Brassica carinata A. Braun) is an economically significant self-pollinating oilseed crop valued for its adaptability and diverse industrial applications. Despite its long cultivation history in Ethiopia and the availability of extensive germplasms, a comprehensive understanding of its diversity is lacking, hindering effective breeding strategies. This study aimed to evaluate the morpho-physiological, seed oil, fatty acid, and genetic diversity of 386 collections of B. carinata accessions. Three independent experiments were conducted. First, 386 accessions were phenotypically characterized for 27 agro-morphological and physiological traits for two successive growing seasons in 2022 and 2023 using an augmented block design, replicated twice. Second, seed oil content and fatty acid profiles were analyzed using Nuclear Magnetic Resonance Spectroscopy (NMRS) and Near-Infrared Reflectance Spectroscopy (NIRS), respectively, to identify significant variation ($P < 0.05$) and promising accessions for industrial and edible oil applications. Third, genetic diversity was assessed in 188 accessions using 3793 DArTSeq-generated single nucleotide polymorphism (SNP) markers. Phenotypic analyses revealed significant variation ($P \leq 0.001$) within accessions for all measured traits indicates substantial phenotypic diversity. Cluster analysis identified four distinct groups with significant inter-cluster divergence, indicating potential for selection. Promising accessions (n = 19) with high seed yields and oil contents were identified. Seed oil content ranged from 37.88% to 46.98%, with high heritability (85-94%) and genetic advance (22.30-59.29%) observed for all traits. Specific accessions (n = 19) were identified as valuable sources of high oil contents and desirable fatty acid profiles. Genetic diversity and population structure analysis using STRUCTURE, principal coordinate analysis (PCoA), and neighbor-joining trees identified two distinct subpopulations with limited genetic differentiation (PhiPT = 0.02) and high gene flow (Nm = 5.74). PCoA indicated low molecular variation, and genetic diversity indices (HE = 0.21, PIC = 0.13) suggested heterozygote deficiency, likely due to restricted cross-fertilization. Accessions did not cluster strictly according to geographical origin, indicating that other factors influence genetic diversity. Higher genetic variation was observed within populations (65.19%) than between populations (44.81%). In conclusion, this study revealed substantial phenotypic variability and medium genetic diversity in the Ethiopian mustard germplasm. These findings highlight the importance of intra-population diversity in breeding programs and suggest that geographic origin is not the primary determinant of genetic diversity. Promising accessions identified for high seed yield, oil content, and desirable fatty acid profiles provide valuable resources for future improvement and commercialization. This study contributes to a more comprehensive understanding of B. carinata diversity, informing efficient conservation and breeding strategies.

Key words: Cluster Analysis; Ethiopian Mustard; Fatty Acids; Genetic Diversity; Germplasm; Heritability; Morpho-physiological Traits; NIRS, NMRS, Oil Content; Principal Component Analysis; SNPs

CHAPTER 1

1. GENERAL INTRODUCTION

1.1. Background and Justification

Brassica crops belong to the *Brassicaceae* family and are among the top ten economically important crops globally (Soengas *et al.*, 2021). This diverse genus encompasses both widely consumed vegetables (cabbage, broccoli, and kale) and important oilseed crops (*Brassica carinata* and rapeseed), cultivated for their nutritional value and industrial applications (MasterClass, 2021; Crop Trust, 2024). Brassicas are rich in fiber, vitamins (C and K), minerals, and antioxidants (Francisco *et al.,* 2017). Epidemiological studies have linked their consumption to reduced risk of various diseases, including cancer and cardiovascular diseases (Baptista *et al.*, 2021). These health benefits are attributed to bioactive phytochemicals such as glucosinolates, phenolics, carotenoids, and ascorbic acid (Borges *et al.,* 2023). Furthermore, oilseed Brassicas, such as *B. carinata* and rapeseed, are major contributors to global vegetable oil production and serve diverse industries, including food, biofuels, and pharmaceuticals (Cavanagh, 2013).

Ethiopia's diverse agro-ecological zones offer significant potential for Brassica crop production, a sector currently underperforming relative to its capacity (Licata *et al.*, 2017). Although cultivation is expanding across arid and semi-arid regions (FAOSTAT, 2019; Gelaye and Tadele, 2022), national exports remain limited, totaling only USD 322,452.88 from cabbage and Ethiopian mustard in 2018 (1,575 and 24.75 tons respectively; Tabor *et al.,* 2022), a stark contrast to global Brassica production (68 million tons; FAOSTAT, 2019), and the associated multi-billion dollar market (Valdivieso *et al.*, 2019). This underperformance is particularly notable considering Ethiopia's substantial annual imports of fuel (USD 5.6 billion) and vegetable oil (USD 576 million) (World Bank Group, 2022). Bridging this gap between potential and realized production requires strategic development of Ethiopia's Brassica sector (Trading Economics, 2023).

Although Ethiopia possesses favorable agro-ecological conditions for Brassica cultivation, production is hampered by variuos constraints that limit yield and quality (Gelaye and Tadele, 2022). These include scarcity of improved cultivars, suboptimal farming practices and technologies, escalating climate change impacts, prevalent pest and disease pressures, and inadequate infrastructure and market access (Wiersinga and Jager, 2009). Addressing this requires a multi-pronged approach: developing high-yielding, stress-tolerant cultivars suitable for diverse applications; improving agronomic practices and pest management; enhancing access to inputs; and investing in infrastructure development (Tabor *et al.*, 2022). Continued research and innovation advancements are crucial for boosting productivity, economic viability, and export potential that facilitate import substitution, product diversification, and job creation within Ethiopia's agricultural sector. Therefore, this study focused on Ethiopian Mustard (*Brassica carinata* A. Braun), a promising oil crop with significant global market potential and diverse industrial applications (World Bank Group, 2022).

Ethiopian mustard, also known as ‘Gomen zer’, ‘Yehabesha gomen’, Ethiopian rape, and Abyssinian mustard, belongs to the *Brassicaceae* family (Mekonen *et al.,* 2014). It is an allotetraploid oilseed crop (BBCC, n=17) with a fascinating evolutionary history (Royal Botanic Garden, 2020) that has arisen through natural interspecific hybridization between the diploid species *B. nigra* (BB, n=8) and *B. oleracea* (CC, n=9), followed by chromosome doubling (Shyam *et al.,* 2012). *Brassica carinata* is believed to have originated in the Ethiopian highlands and surrounding regions of East Africa and the Mediterranean coast

(Simmonds, 1979). With a cultivation history in Ethiopia dating back to approximately 4000 BC, it is one of the oldest cultivated crops (Alemayehu and Becker, 2002). This species is primarily propagated through seeds and is predominantly self-pollinating (Iboyi *et al.,* 2021), although cross-pollination with other Brassica species can occur in the presence of insect pollinators (Seepaul *et al.,* 2021). This annual crop exhibits a determinate growth habit, with an erect stature reaching heights of up to 1.4 meters (Gan, 2007). Its well-developed taproot and extensive rooting systems enable efficient acquisition of nutrients and moisture (Zanetti *et al.,* 2012). The adaptability of *B. carinata* to diverse environments, thriving in areas with annual rainfall between 700 and 1000 mm and temperatures ranging from 15 to 20 °C, has facilitated its spread beyond its native range (Tesfaye *et al.,* 2023). It is now cultivated in parts of South and North America, where it is increasingly utilized for biofuel production (George *et al.,* 2021).

Brassica carinata is of significant cultural and economic importance in Ethiopia (Rahiel *et al.,* 2024), with over three million smallholder farmers cultivating it for versatile applications (Alemayehu and Becker, 2002). Traditionally, its leaves and young stems are consumed as vegetables, while ground seeds are used to lubricate "injera" baking pans (Abebe *et al.,* 2009). The seed oil serves multiple purposes, including as a seasoning for spicy foods, lighting and medicinal uses (MoA, 2018). Beyond its traditional uses, *B. carinata* has emerged as a multifaceted crop with a wide array of applications offering significant benefits in nutrition, health, industry, environmental sustainability, and climate change mitigation (Rahiel *et al.,* 2020). Rich in nutrients, particularly glucosinolates and polyphenols, *B. carinata* has demonstrated potential as a cancer-preventive food. Both raw and cooked forms have been shown to reduce DNA damage caused by carcinogens such as aflatoxin B1 (Kumar *et al.,* 2021). The presence of allyl isothiocyanate in raw *B. carinata* contributes to its anti-genotoxic properties, further emphasizing its value in promoting a healthy diet (Zanetti *et al.,* 2012). This diverse range of applications underscores the importance of *B. carinata* as a crop with significant potential for both traditional and modern use, spanning from local culinary practices to global health and environmental concerns.

In addition, *B. carinata* is suitable for diverse, non-food industrial applications (George *et al.,* 2021) owing to its high oil content, particularly erucic acid (31–46%). These applications include the production of biofuels, high-value chemicals, and sustainable aviation fuels (Seepaul *et al.,* 2021). Moreover, its oil has applications in oleochemical industries, serving as a slip agent in plastic films, lubricants, and detergents (Schulmeister *et al.,* 2019). The seed meal and hulls are valuable resources utilized in animal feed, biofertilisers, and bioenergy production, enhancing the overall sustainability and economic viability of the crop (Walelign *et al.,* 2022). The cultivation of *B. carinata* contributes to environmental sustainability by improving soil health and reducing soil erosion (Mekuria *et al.,* 2018). As a winter crop, it helps retain soil moisture and organic matter, while minimizing nutrient loss (Bulcha *et al.,* 2017). Its ability to suppress weeds further enhances ecological benefits (Bulcha *et al.,* 2007).

Moreover, *B. carinata* has been recognized for its potential to mitigate greenhouse gas emissions (Tesfaye *et al.,* 2023). When used as a feedstock for biofuels, their cultivation can significantly reduce carbon intensity compared to fossil fuels (Basili and Rossi, 2018). The adaptability of the crop to diverse climatic conditions allows for integration into various cropping systems, contributing to climate resilience in agriculture (Seepaul *et al.,* 2021). Studies have shown the significant promise of *B. carinata* as a rotational and intercropping partner with various food crops that share similar ecological requirements (Zegada *et al.,* 2011). It excels as a cover crop, mitigates soil erosion, reduces herbicide use, and promotes nutrient balance (Pane *et al.*, 2013). Furthermore, the unique resilience of *B. carinata* to harsh environments, diseases, and pests allows it to thrive in marginal lands with high seed yields and large seed sizes (Chavan and Kamble, 2014). Generally, *B. carinata* is a versatile and valuable crop with a wide range of applications that contribute significantly to the economic, social, and environmental well-being of communities, particularly in Ethiopia (Lal *et al.,* 2019). Its diverse benefits, spanning from nutritional and health aspects to industrial applications and environmental sustainability, solidify its position as a vital resource for sustainable development (Rahiel *et al.,* 2020; Iboyi *et al.,* 2021).

The cultivation of *B. carinata* in Ethiopia has remained predominantly traditional for centuries, primarily conducted by smallholder farmers on small plots of land, as border plants on cereal farms, or intercropped (Yared, 2011). However, areas above 1500 meters above sea level in various parts of the country have been reported to be highly suitable for *B. carinata* cultivation. In research fields, its productivity has been reported as 20–30.3 t ha^{-1} for seed yield, 106.2–112.1 L ha^{-1} for oil yield, 37–45.6% for oil content, and 31–46% for erucic acid content (Misteru *et al.,* 2013; Bulcha and Adugna, 2017). The balanced composition of unique monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), and saturated fatty acids (SFAs) in oil has attracted global interest and gained significant market value (Ghosh and Bauri, 2018; Rahiel *et al.,* 2020). Despite limited empirical data from Ethiopia, global market analyses have reported significant growth, particularly in Asia-Pacific and North America, with a market value reaching $34,164.9 million in 2023 (Chemanalyst, 2024). Owing to these facts, large-scale *B. carinata* seed production, oil extraction, and blending with soybean oil for human consumption and exports have been initiated by various local and international investors in Debre Elias and other areas of the country (personal communication). Additionally, Ethiopia is the center of origin for *B. carinata*, with over 400 germplasms maintained at the Ethiopian Biodiversity Institute (EBI) (http://www.ebi.gov.et) and Ethiopian Institute of Agricultural Research (EIAR) (http://www.eiar.gov.et) gene banks. These germplasms represent a rich source of genetic diversity, which is crucial for its traits improvement.

Despite its significant market potential and extensive germplasm resources, Ethiopia's production of *B. carinata* has been hampered by a critical shortage of technological advancements for its production and improvement. Although national breeding programs initiated four decades ago and released six varieties (Yellow Dodola, Tesfa, Derash, Awassa pop, Holetta-1, and S-67) through pedigree selection (Alemayehu and Becker, 2002; Bulcha *et al.*, 2007), the genetic diversity and full productive potential of the crop remain largely unexplored. This knowledge gap necessitates further research on innovative strategies to optimize this valuable oilseed crop.

Plant genetic resources are critical for global food security and livelihoods (Haussmann *et al.*, 2006). Various morphological, biochemical, and molecular markers have been used to characterize the genetic diversity of plants (Agong *et al.*, 2000; Afzal *et al.*, 2016; Syed *et al.*, 2019). Genetic diversity underpins adaptation and evolution and enables the development of superior varieties (Azeez *et al.*, 2018). Effective conservation strategies require a combined ex situ and in situ approach (Karp, 1997) to maximize the genetic resources available for breeding programs and to ensure sustainable production. Therefore, assessing the genetic diversity of *B. carinata* is crucial for understanding its existing variability and developing efficient collection, conservation, and breeding strategies (Hawkes, 1983; Lee *et al.*, 2020). Systematic germplasm characterization using morphological, physiological, nutritional, biochemical, and molecular marker data (Yun *et al.*, 2020) can unlock the potential of existing diversity, broaden the genetic base, and improve yield and related traits (Mehmood *et al.,* 2008; Nguyen and Norton, 2020). This knowledge is vital for developing resilient, high-yielding varieties to meet the future food and energy demands.

Among the diverse approaches, morphological characterization is a fundamental technique for assessing genetic diversity, providing a cost-effective and readily accessible method for plant classification and germplasm collection (Upadhyaya *et al.*, 2008; Kachare *et al.*, 2020; Mwakha *et al.,* 2020). Its simplicity and rapid scoring make it valuable for evaluating the traits of crop plants (Singh *et al.,* 2018). This approach typically involves recording both qualitative and quantitative traits, which are then analyzed using univariate and multivariate statistical methods, such as cluster and principal component analysis, to identify relationships among accessions (Aremu, 2005; Bonny *et al.*, 2019; Malek *et al.*, 2014). The effectiveness of morphological characterization has been proved across diverse species and geographic locations, including soybean (Iqbal *et al.*, 2008), rice (Rashid *et al.*, 2008), Lactuca (El-Esawi, 2008), cauliflower and broccoli (Kop *et al.,* 2003), winter squash (Balkaya *et al.,* 2010), and Ethiopian mustard (Rahiel *et al.*, 2024). However, further research is needed to fully exploit the potential of morphological characterization to understand genetic diversity within specific crops and regions.

Although preliminary studies in Ethiopian (Nigussie & Becker, 2002; Yared, 2011; Misteru et al., 2013; Tesfaye *et al.,* 2014; Rahiel *et al.,* 2024) have documented significant morphological variation in *B. carinata* accessions, their reliance on limited quantitative agro-morphological traits hinders a comprehensive understanding of the crop's germplasm diversity. This limitation stems from several factors: the restricted number of easily quantifiable traits; the potential for low heritability of some traits; and the confounding effects of the environment, plant age, cultivation practices, and underlying genetic architecture on trait expression (Blazakis *et al.,* 2017; El-Esawi *et al.,* 2012). Although morphological characterization provides a valuable initial assessment of diversity and aids in landrace identification for breeding programs (Panwar *et al.,* 2013), a more robust approach is needed. To fully characterize *B. carinata* germplasm variability, a multifaceted analysis integrating morphological, physiological, biochemical, and molecular techniques is essential for generating reliable and comprehensive results, thereby informing the development of more effective breeding strategies and maximizing the utilization of this crop's genetic potential.

Understanding the morpho-physiological diversity within *B. carinata* germplasm is crucial for effective crop improvement (Weerakoon and Somaratne, 2010). This knowledge will enable researchers to identify promising accessions with desirable traits, inform strategic conservation efforts, and lay the groundwork for the development of cultivars tailored to specific environments and applications. Studies on related Brassica species, such as *B. napus* (Muhammad and McNeilly, 2004), canola (Roubina and Muhammad, 2006), and cabbage (Albino and Giancarlo, 2005), have documented significant morpho-physiological variation; however, a comprehensive analysis of *B. carinata* diversity remains limited. This is despite preliminary findings that have revealed exploitable genetic variability in seed oil content and fatty acid composition (Genet *et al.,* 2005; Yared *et al.,* 2011; Fekadu, 2021). Therefore, a detailed analysis of the morpho-physiological traits, seed oil content, and fatty acid profiles across a broad collection of *B. carinata* germplasms is warranted. Such a study would provide critical insights for developing improved cultivars with enhanced oil quality and quantity tailored to specific industrial demands (Kanchan *et al.,* 2021) and ultimately unlock the full potential of this valuable oilseed crop.

In addition, DNA-based molecular markers offer a more robust approach for detailed genetic characterization (Li *et al.,* 2022), and previous studies on *B. carinata* have employed a range of markers with varying levels of resolution. These studies utilized early stage techniques, including Random Amplified Polymorphic DNA (RAPD) (Peng *et al.,* 2023) and Amplified Fragment Length Polymorphism (AFLP) (El-Esawi *et al.*, 2016), and more advanced markers such as Simple Sequence Repeat (SSR) (Thakur *et al.,* 2021) and Single Nucleotide Polymorphism (SNP) (Tesfaye *et al.*, 2023). Despite their advantages, these markers often suffer from limitations such as low density and incomplete coverage (Thakur *et al.,* 2019; Khedikar *et al.,* 2020), hindering comprehensive genetic diversity analyses. To address these limitations, the Diversity Array Technology (DArT) emerged in the early 2000s (Valdisser *et al.,* 2017).

Advancements in high-throughput DArTSeq SNP marker technology have revolutionized plant genetic diversity and population structure studies (Mijangos *et al.*, 2022). These markers are abundant throughout the genome, easily replicable, reliable, and capable of enabling genome-wide coverage and large-scale genotyping (Fiust *et al.*, 2015). Although DArTSeq SNP markers have been successfully applied to crops, such as sorghum (Muhammed *et al.,* 2023; Phoebe *et al.*, 2023), barley (Matties *et al.,* 2012), wheat (Laido *et al.,* 2013), macadamia (Alam *et al.*, 2018), and maize (Adu *et al.,* 2019), their application in *B. carinata* diversity studies remains unexplored. The lack of robust genome-wide data has restricted previous *B. carinata* studies to parent selection based on phenotypic characteristics or limited old molecular marker data, which has led to limited estimation of the true extent of genetic diversity and inaccurate population structure inference within and among *B. carinata* populations. This constraint has significantly hindered the implementation of large-scale genotyping initiatives, which are essential for the comprehensive assessment of genetic diversity and population structure across a broad spectrum of accessions. This presents a significant research gap, as the utilization of DArTSeq SNP markers could provide a more comprehensive and accurate assessment of *B. carinata* genetic diversity, facilitate the development of more effective breeding strategies and the utilization of the full genetic potential of this valuable crop.

In general, the underutilization of *B. carinata's* potential stalks mainly from the limited understanding of its genetic diversity. This knowledge deficit constrains the development of improved cultivars suited to varied environments and end uses. Addressing this requires a comprehensive research strategy that integrates morphophysiological, seed oil, fatty acid composition, and molecular analyses. Such a multifaceted approach will provide a robust assessment of genetic variability, informing future conservation and breeding programs to enhance the contribution of *B. carinata* to the traditional and emerging modern production, processing, and export sectors in Ethiopia. Therefore, this study was undertaken with the following general and specific objectives.

1.2. Research Objective

1.2.1. General Objective

- To generate knowledge and contribute to the improvement and conservation of mustard agro-biodiversity in Ethiopia through a comprehensive assessment integrating morpho-physiological, seed oil compositional, and genetic diversity.

1.2.2. Specific Objectives

- To quantify the phenotypic diversity within Ethiopian mustard germplasm through comprehensive quantitative assessment of morph-physiological traits

- To estimate the extent of variation in seed oil content and individual fatty acid profiles across Ethiopian Mustard germplasm
- To assess the genetic diversity and population structure of Ethiopian mustard germplasm using DArTseq SNP markers

1.3.Thesis Organization

This thesis is composed of five distinct chapters, each of which addresses a specific objective outlined above. Although these chapters are interconnected and presented as independent research papers, there may be some overlap in content and references. The organization of the chapters are as follows:

Chapter	Title
1	General Introduction
2	Analyzing the Diversity within Ethiopian Mustard (*Brassica carinata* A. Braun) Germplasm for Quantitative Agro-Morphological and Physiological Traits
3	Evaluating the Variability in Seed Oil Content and Fatty Acid Composition of Ethiopian Mustard (*Brassica carinata* A. Braun) Germplasm
4	Estimating Genetic Diversity and Population Structure in Ethiopian Mustard (*Brassica carinata* A. Braun) Germplasm using DArTseq-generated High-Density SNP Markers
5	Summary and Recommendations

CHAPTER 2

2. Analyzing the Diversity within Ethiopian Mustard (*Brassica carinata* A. Braun) Germplasm for Quantitative Agro-Morphological and Physiological Traits

Abstract

Ethiopian mustard (Brassica carinata A. Braun) is a versatile oil crop cultivated for vegetable and oilseed production. Despite the availability of numerous landraces, a comprehensive understanding of their phenotypic diversity remains limited, hindering breeding efforts to improve the crop. This study evaluated 386 Ethiopian mustard accessions for 21 agro-morphological and six physiological traits over two growing seasons in 2022and 2023 using an augmented block design. This comprehensive analysis aimed to uncover the extent of phenotypic variation within the germplasm collection and identify promising accessions with desirable traits for breeding. Significant variation (P $\leq$ *0.001) was observed among accessions for all studied traits, revealing substantial phenotypic diversity within the germplasm collection. Principal component analysis identified key contributors to this variability, with the top five components explaining 61.23% of the total variation. Cluster analysis grouped accessions into four distinct clusters, with the highest inter-cluster divergence (18.21) between clusters 3 and 4, highlighting the potential for selecting diverse accessions across phenotypic groups. Cluster 2 exhibited the highest intra-cluster distance (6.71) and mean genetic distance (5.23), implying extensive agro-morphological and physiological variability, valuable for breeding to develop cultivars with diverse traits. Several superior performing accessions, including acc-192, acc-386, acc-1, acc-11, acc-197, acc-377, acc-235, acc-294, acc-302, acc-112, acc-331, acc-152, acc-55 and acc-72 were identified based on their high seed yield and oil content. These promising candidates hold potential for further improvement and incorporation into breeding programs. Overall, these findings reveal extensive genetic diversity in Ethiopian mustard and provide valuable insights into future breeding strategies, highlight the importance of conserving genetic resources to enhance crop performance, adaptability, and versatility for sustainable agriculture and alternative energy initiatives.*

Keywords: Agro-morphological traits; Ethiopian Mustard; Germplasm; Heritability; Phenotypic diversity; Physiological traits; Principal Component Analysis

2.1. Introduction

Ethiopian mustard (*Brassica carinata* A. Braun), also known as ‘Gomen zer’, ‘Yehabesha gomen’, Ethiopian rape, and Abyssinian mustard, is a vital oilseed crop with a 4000-year cultivation history in Ethiopia (Misteru *et al.*, 2013). The crop is a self-pollinating annual plant belonging to the *Brassicaceae* family (Bulcha and Adugna, 2017). It demonstrates remarkable adaptability to diverse ecological conditions and plays a crucial role in Ethiopian agriculture, supporting over three million smallholder farmers (Yared, 2011; Mekonen *et al.,* 2014). This crop is the third most important oilseed crop in Ethiopia, following nigerseed and flaxseed, with a total yield of 74,766.6 tons produced over 45,167.81 hectares (FAOSTAT, 2019).

In Ethiopia, *B. carinata* holds significant cultural and economic value (Rahiel *et al.*, 2024). Its ground seeds are traditionally used to lubricate 'injera' baking pans, wheras young leaves serve as a vegetable relish (Abebe *et al.,* 2009). The seeds also possess medicinal properties and are used in traditional beverages (MoA, 2018). The oil extracted from *B. carinata* seeds has substantial industrial potential, primarily for biofuel production, owing to its high erucic acid content (Rouillard, 2009). Furthermore, *B. carinata* serves as an excellent rotational and intercropping partner for various food crops, contributing to improved soil health and productivity (Zegada and Monti, 2011; Basili and Rossi, 2018; Lal *et al.,* 2019). As a cover crop, it mitigates soil erosion, reduces herbicide use, and promotes a nutrient balance (Pane et al., 2013). Its resilience to harsh environments and pests makes it suitable for cultivation on marginal lands (Purakayastha *et al.,* 2008; Chavan and Kamble, 2014). The combination of its agricultural versatility, industrial applications, and environmental benefits makes *B. carinata* a promising candidate for addressing contemporary challenges in food security, sustainable agriculture, and renewable energy production. These multifaceted attributes have attracted the global attention for *B. carinata* as a potential crop for sustainable agriculture and alternative energy development (Rahiel *et al.*, 2020).

Ethiopia has a diverse collection of over 400 *B. carinata* accessions, maintained at the Ethiopian Biodiversity Institute (EBI), along with released varieties at the Holetta Agricultural Research Center (HARC) (EBI, 2022; HARC, 2022). This rich germplasm pool provides significant opportunities for the development of superior cultivars with enhanced seed yield, oil quality, and secondary metabolite content. However, a comprehensive understanding of the phenotypic variability within this collection remains limited, which impedes effective germplasm management and cultivar improvement. This knowledge gap underscores the need for comprehensive characterization of the available gene pool to fully exploit the potential of the crop in breeding programs aimed at developing new cultivars with enhanced seed yield and oil quality traits. Such characterization is crucial for identifying and selecting desirable traits, understanding genetic diversity, and guiding targeted breeding efforts to meet agricultural and industrial needs. Multiple approaches, including morphological, physiological, biochemical, and molecular marker techniques, have been reported for comprehensive diversity studies; however, morpho-physiological diversity analysis remains the primary method for initial germplasm characterization (Smith, 1984).

The rich morpho-physiological diversity in plants, including *B. carinata,* is the result of centuries of cultivation, selective breeding, and natural variation, and represents a valuable resource for crop improvement (Muthoni, 2010). This approach provides a cost-effective and readily accessible means for assessing diversity, particularly in resource-limited settings. Moreover, morpho-physiological traits are often directly related to agronomic performance and adaptability, making them valuable indicators for breeding programs. This characterization serves as a foundation for more advanced genetic studies and targeted improvements. Systematic evaluation of morpho-physiological traits across the *B. carinata* germplasm collection can reveal patterns of variation, identify promising accessions, and inform strategic decisions in breeding programs.

Recent studies have successfully employed various analytical techniques to assess the agro-morphological and physiological diversity of *Brassica* species, including classification and regression tree algorithms (Weerakoon and Somaratne, 2010) and morpho-physiological trait analyses (Kanchan *et al.,* 2021). Although previous studies have provided valuable insights into the morphological variation of *B. carinata* (Nigussie and Becker, 2002; Yared, 2011; Misteru *et al.,* 2013; Tesfaye *et al.,* 2014; Rahiel et *al.,* 2024), they have been limited in scope, focusing primarily on a small number of accessions (36–64) and solely on morphological traits which do not provide a holistic picture of the genetic potential of *B. carinata*. Hence, a more comprehensive understanding of both morphological and physiological diversity in broader germplasm collections is necessary to unlock the full potential of this valuable crop. To address this knowledge gap, the present study was designed to systematically employ a combination of morphological and physiological diversity analysis techniques and evaluate 386 Ethiopian mustard accessions for both quantitative agro-morphological and physiological traits. This comprehensive analysis aimed to determine the extent of phenotypic variation within the germplasm collection across multiple quantitative traits, identify promising accessions with desirable traits for breeding, inform strategic conservation efforts, and lay the groundwork for developing improved cultivars tailored to diverse environments and applications. These findings are expected to provide a holistic picture of the genetic potential of *B. carinata* and contribute significantly to efficient germplasm management and exploitation, ultimately supporting sustainable agriculture and alternative energy development initiatives.

2.2. Materials and Methods

2.2.1. Description of Experimental Site

The experiment was conducted from June–December in 2022 and 2023 at Holeta Agricultural Research Center (HARC) during the main cropping season. HARC is located 30 km southwest of Addis Ababa at 9°06′ N, 38°31′ E, and sits 2400 m above sea level. The average maximum and minimum temperatures are 22 and 6 °C, respectively with a rainfall of 1100 mm. The center has a red-brown fertile soil with a pH range of 6.0 –7.5 (Nigussie and Mesfin, 1994).

2.2.2. Plant Materials

The experiment evaluated a total of 386 *B. carinata* accessions collected from diverse growing regions across Ethiopia (Figure 2.1) between 1984 and 2022 by EBI and HARC. These accessions included 339 accessions obtained from EBI, 36 accessions and five released varieties (S-67, Yellow Dodolla, Holletta-1, Derash, and Tesfa) sourced from HARC and the remaining six accessions were collected by researchers. Following the identification of the collection areas in each zone, accessions were systematically collected across a range of altitudes to capture altitudinal diversity and ensure sample representativeness. Passport data for the accessions are available in the appendix Table 2.1.

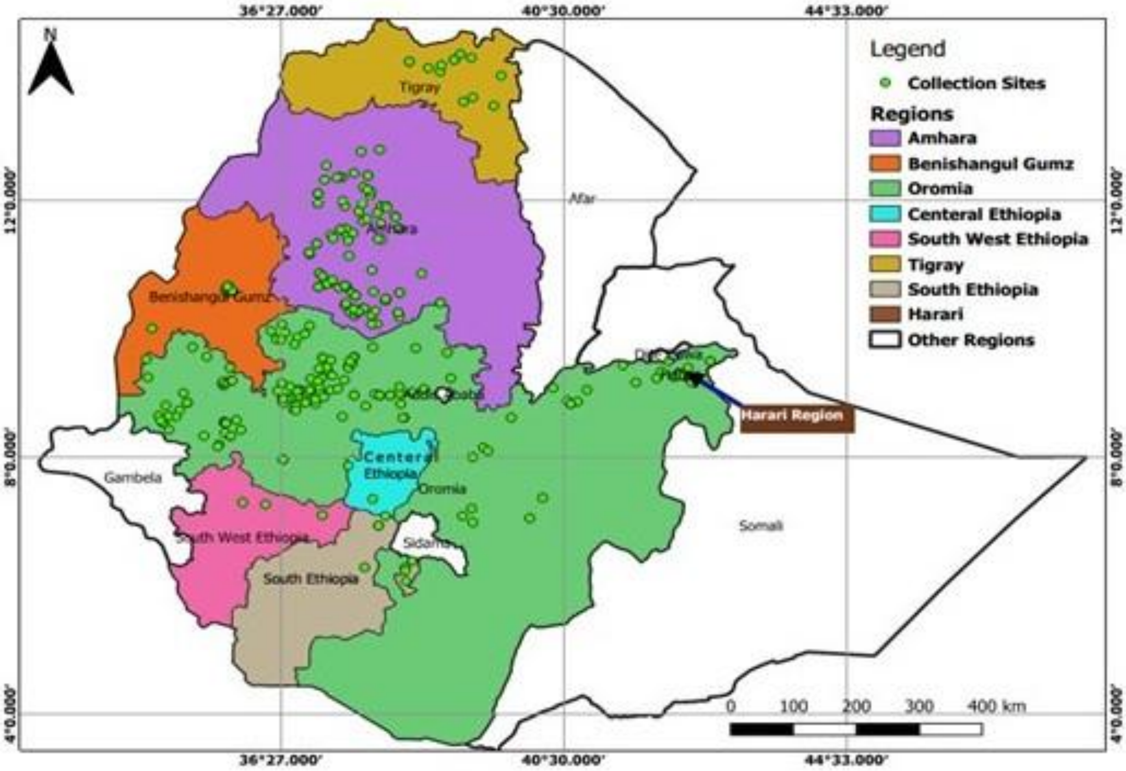

Figure-2.1 Geographic distribution of *B. carinata* accessions used in the study

2.2.3. Experimental Set-up, Design and Management

The field experiment was conducted following the established national guidelines for *B. carinata* cultivation (http://www.eiar.gov.et). The selected site was subjected to three deep (30 cm) tills and disc harrowing using a tractor. The layout was implemented using a 3m x 4m x 5m triangular system. The experiment was laid out using an augmented block design (ABD) with four main blocks each 3.6 m × 45 m, replicated twice, and 2 m pathways separating them. The main blocks were divided into two sub-blocks, each with 1.5 m long double ridge plots. To ensure unbiased evaluation, the positions of the accessions and check varieties were randomized. Individual accessions were randomly assigned to plots and planted at a spacing of 10 × 30 cm between the plants and ridges. Checks were sown after every 10 accessions. Seeds were hand-drilled at a rate of 10 kg/ha, and fertilizers were applied at rates of 46 kg/ha N and 69 kg/ha P_2O_5 following established national guidelines for *B. carinata* cultivation (http://www.eiar.gov.et). Standard agronomic practices were employed throughout the experiment to ensure consistency and uniformity, as described by Adefris (2005).

2.2.4. Data Collection

Data on 21 agro-morphological and six physiological traits were collected from ten randomly selected *B. carinata* plants per plot (mid-row). Trait selection and measurement followed the International Board for Plant Genetic Resources guidelines for Brassica and Raphanus descriptors (IBPGR, 1990). Agro-morphological traits were recorded at various stages of the plant's growth cycle, while physiological measurements were taken on fully expanded leaves (4.1 cm^2) during the vegetative stage at various times of day using appropriate portable instruments for each parameter. Oil content was analyzed using nuclear magnetic resonance spectroscopy (NMRS) equipment (Newport 4000, UK) following the protocols established by the Oregon State University Seed Laboratory (https://seedlab.oregonstate.edu/). Detailed information about the trait types, their corresponding codes, measurement times, and tools, along with their respective units of measurement, is provided in Table 2.1.

Table 2.1: Agro-morphological and physiological traits, their abbreviations and descriptors used in the study

Trait	Code	Descriptor
Days to Emergence	DE	Number of days from seeding to 90% seedling emergence
Petiole Length	PL	Measured in cm from stalk-stem attachment to leaf blade base
Number of Leaves	NL	Number of leaves per plant
Leaf Length	LL	Measured in cm from base to tip
Leaf Diameter	LD	Measured the diameter in cm across the mid-point of the leaf blade
Number of Primary Branches	NPB	The average number of primary branches per plant.
No. Secondary Branches	NSB	Number of secondary branches per primary branch
Days to Flowering	DF	Time from sowing to 90% flower emergence
Flower Inflorescence Length	FIL	Measure in m from primary branch base to main flower tip at full bloom
Days to Harvest	DH	Time from sowing to harvest
Plant Height	PH	Measured in m from ground surface to main stem tip at harvest
Diameter of Stem	DS	Measured in mm on main stem at harvest.
Silique Number	SN	Number of pods per plant at harvest

Silique Length	SL	Measure pod length in cm at harvest
Silique Diameter	SD	Measure pod diameter in mm at mid-point at harvest.
Number of Seed/Silique	NSS	Number of seeds per pod at harvest
Number of Seed/Plant	NSP	Number of seeds per plant at harvest
1000 Seeds Weigh	TSW	The weight in g of 1000 seeds sampled from each plot using 0.01g precision balance.
Seed Yield	SY	Seed yield per plot (t/ha), after dried to 7% moisture content
Oil Content	OC	Proportion of seed oil to dry seed weight in % measured by NMRS.
Oil Yield	OY	Amount of oil (L), obtained by multiplying seed yield per plot with its corresponding oil content
Number of Stomata	NS	Number of stomata/mm^2 of a leaf at early morning using 100x magnifying light microscope
Width of Stomata	WS	Stomata pore length (µm) of a leaf at early morning using 400x magnifying light microscope
Photosynthesis Rate	PR	CO_2 fixed on a leaf (µmol m^{-2} s^{-1}) at mid-morning using CIRAS-3 portable photosynthesis system
Transpiration Rate	TR	water loss in mL on a leaf at mid-morning using CIRAS-3 portable photosynthesis system machine
Stomata Conductance	SC	CO_2 influx/water vapor efflux (mmol CO_2 m^{-2} s^{-1}) on a leaf at mid-morning using porometer
Chlorophyll Fluorescence	CF	The maximum quantum yield of photosystem II (PSII) in fluorescence unit (FU) at early morning measured by handheld fluorometer, indicate plant stress response to environmental factors.

2.2.5. Data Analysis

Prior to conducting the analysis of variance (ANOVA), the data were checked for adherence to ANOVA assumptions using SAS 9.4 (SAS Institute, 2023). As the yearly ANOVA showed that year had no significant effect ($p > 0.05$) on any agro-morphological or physiological trait, data from both years were combined for subsequent analyses.using the same package at a significance level of $\alpha = 0.05$. To address potential data scale discrepancies, the combined means were standardized (mean = 0, variance = 1) using R 4.3.2 (R Development Core Team, 2023).

Variance component analysis was conducted to understand the extent of genetic and environmental influences on the variation within the accessions, the phenotypic and genotypic variances were calculated using the variability package (Popat *et al.,* 2020) in R. Furthermore, the genotypic and phenotypic coefficients of variations were computed to assess the magnitude of variability relative to the mean, using the following formulas as described by (Kwon and Torrie, 1964; Falconer, 1981; Singh and Chaudhary, 1985; Syukur *et al.,* 2012). $\sigma^2{}_G = \frac{MS_G - MS_{GxE}}{rl}$, where $\sigma^2{}_G$ = genotypic variance, MS_G = mean square due to accession, MS_{GxE} = mean square due to accession by Environment interaction, r = number of replication.$\sigma^2{}_P = \sigma^2{}_G + \frac{\sigma^2{}_{GxE}}{rl} + \frac{\sigma^2{}_E}{rl}$, where $\sigma^2{}_P$ = phenotypic variance, $\sigma^2{}_G$ = genotypic variance, MS_{GxE} = mean square due to accession by Environment interaction, $\sigma^2{}_E$ = Environmental variance, r = number of replication.

$GCV = \frac{\sqrt{\sigma^2{}_G}}{\bar{x}} \times 100$, where $GCV =$ Genotypic Coefficient of variation $\sigma^2{}_G$ = genotypic variance, $\bar{x}$ = population mean of the quantitative character.

$PCV = \frac{\sqrt{\sigma^2{}_P}}{\bar{x}} \times 100$, where $PCV =$ Phenotypic Coefficient of variation, $\sigma^2{}_P$ = phenotypic variance, $\bar{x}$ = population mean of the quantitative character.

To assess the proportion of phenotypic variance attributable to genetic factors, broad-sense heritability ($H_b{}^2$) was calculated for all traits according to the formula detailed by Allard (1960). $H_b{}^2(\%) = \frac{\sigma^2{}_G}{\sigma^2{}_P} \times 100$, where $H_b{}^2 =$ broad-sense heritability, $\sigma^2{}_G$ = genotypic variance, $\sigma^2{}_P$ = phenotypic variance. To estimate the potential improvement in traits through one cycle of selection, the expected genetic gain was predicted. This prediction assumed a 5% selection intensity (K) and was calculated using the formula as described by Johnson et al. (1955). $GA = K\,(\sigma_P)\,H_b{}^2$, where GA = genetic advance, K = selection intensity (2.06 at 5%), σ_P = the phenotypic standard deviation of the character, $H_b{}^2 =$ broad-sense heritability. To facilitate the comparison of predicted genetic gains across different traits, genetic advance as a percentage of the mean (GAM) was calculated using the following formula: $GAM\ (\%) = \frac{GA}{\bar{x}} \times 100$, where GAM = genetic advance as a percentage of the mean, GA = genetic advance, $\bar{x}$ = population mean of the quantitative character. Genotypic and phenotypic correlation coefficients for all possible comparisons were also computed using the META-R software (Alvarado *et al.*, 2015).

Multivariate principal component analysis (MPCA) and cluster analysis were conducted using the FactoMineR package (Le *et al.*, 2024) in R to identify key traits and patterns. The corrplot package in R was used to assess the strength and direction of relationships between traits. The unweighted pair group method with arithmetic mean (UPGMA) with agglomerative hierarchical clustering based on Euclidean distances was employed to group accessions, as described by Lance and Williams (1967). Euclidean distances were calculated using the Factoextra package in R (Kassambara, 2022), to determine genetic distances between accessions as described by Gan *et al.* (2007).

2.3. Results and Discussion

2.3.1. Analysis of Variance

2.3.1.1. Variability Based on Univariate Statistics

Analysis of variance (ANOVA) indicated highly significant ($p < 0.001$) variation in most morpho-physiological traits examined (Table 2.2). This signifies the existence of a rich phenotypic diversity within the *B. carinata* germplasm. This genetic richness not only offers immediate breeding opportunities but also represents a valuable resource for long-term crop improvement and adaptation to future challenges. These results align with the findings of Misteru *et al.* (2013), Tesfaye *et al.* (2014), and Takele (2022), all reported significant variation in the agro- morphological traits of characterized *B. carinata* accessions. However, the current study's larger sample size and broader range of examined traits provide a more comprehensive picture of the species' diversity.

Moreover, univariate analysis revealed highly significant ($p \leq 0.001$) variation in most of the agro-morphological and physiological traits (Table 2.2), such as the number of siliques, silique diameter, oil content, stomatal number, and width exhibited minimal variation ($p > 0.05$) due to replication. This minimal variation suggests the effectiveness of blocking design in mitigating seasonal and field-related effects. The successful minimization of environmental influences through proper experimental design provides reliable data for breeders to identify superior accessions. Traits that are less susceptible to environmental influences are typically more heritable and predictable in breeding programs. These results highlight the reduced environmental influence on the collected data, enhancing their reliability in representing the accession performance. Conversely, traits such as days to harvest, plant height, number of seeds per silique, number of seeds per plant, seed yield, oil yield, photosynthesis rate, and stomatal conductance showed non-significant ($p > 0.05$) variation within replicates due to blocking, further confirming the importance of blocking in reducing field variability. These findings are consistent with those of previous reports on *B. juncea* (Kanchan *et al.*, 2021) which documented reduced within-study variation attributed to unpredictable environmental and climatic factors. The observed patterns of trait variation provide valuable insights into breeding programs for *B. carinata*. Traits exhibiting high heritability and low environmental influences, such as silique characteristics, seed yield and oil content, may be more suitable targets for direct selection. In contrast, traits showing greater environmental sensitivity, such as days to seedling emergency, days to flowrinng, transpiration rate and stomatal conductance parameters, may require more complex selection strategies or multi-environmental trials to ensure stable performance across diverse conditions.

This study also revealed strong correlations between the analyzed traits and *B. carinata* performance, with coefficients of determination (R^2) reaching up to 99.8% for chlorophyll fluorescence (Table 2.2). Even the lowest observed R^2 value of 68.65% for days to flowering demonstrated substantial explanatory power. These high R^2 values suggest that the selected traits effectively capture the variation within the *B. carinata* population, providing breeders with reliable indicators for selecting desired characteristics. The results align with the findings of Montgomery (2005) who reported that high R^2 values (approaching 100%) indicate robust variability within a population. Additionally, all measured traits exhibited coefficients of variation (CVs) below the established critical threshold of 30% (Table 2.2), suggests effective control of experimental variability. Notably, the minimum CV observed was a mere 2.61%, further indicated the limited influence of uncontrolled factors on the experimental outcomes. These consistently low CV values across all traits underscore the reliability and reproducibility of the data, providing a solid foundation for informed breeding decisions. These results support those of Gomez and Gomez (1984) who reported that CVs below 30% are acceptable for agricultural field experiments. In general, the combination of high R^2 values and low CVs observed in this study highlights the robustness of the experimental design and the significance of the selected traits in characterizing *B. carinata* accessions. These results not only validate the choice of traits for evaluation but also provide valuable insights into the genetic diversity present within the studied population. Such information is crucial for developing targeted breeding strategies aimed at improving yield, oil content, and other agronomically important traits in *B. carinata.*

2.3.1.2. Variation Based on Range and Mean Values

Analysis of the *B. carinata* accessions revealed substantial variation in both means and ranges across a diverse array of agro-morphological and physiological traits (Table 2.2). This extensive phenotypic diversity revealed rich genetic potential within the evaluated germplasm collection. Notably, seed yield, a critical agronomic trait, demonstrated remarkable variation, ranging from 11.75 to 12.40 t/ha, with a mean yield of 12.08 t/ha. This high average yield suggests the presence of promising lines within the germplasm collection, offering significant potential for breeding cultivars with enhanced seed-production capabilities. The study also revealed considerable variation in six key traits: oil yield (mean: 2.71 t/ha; range: 2.65–2.77 t/ha), days to harvest (mean: 168.94 d; range: 165.52–172.36 d), days to flowering (mean: 117.07 d; range: 81.0–140.0 d), number of seeds per silique (mean: 750.9; range: 70.2–3460), oil content (mean: 43.28%; range: 37.88–46.98%), and silique number per plant (mean: 99.99; range: 15.1–546.1) (Table 2.2). The extensive range observed in days to flowering and harvest is particularly noteworthy as it indicates the potential to develop varieties suited to diverse planting windows and adaptable to varying environmental conditions. This flexibility in phenology could prove invaluable in breeding cultivars that are resilient to climate variability and are suitable for different agricultural systems. The substantial variation in seed number per silique and silique number per plant suggests opportunities for increasing the overall seed yield through targeted breeding efforts. Similarly, the range in oil content (37.88–46.98%) presents an opportunity to develop superior varieties with improved oil yield per hectare. This rich agro-morphological diversity within the germplasm collection represents a valuable resource for *B. carinata* improvement. It provides breeders with a robust foundation for targeted enhancements in seed and oil yields, earlier flowering and maturation times, increased seed and silique production, and the development of cultivars with enhanced environmental adaptability. These findings align with previous reports on *B. carinata* (Muthoni, 2010; Yared *et al.*, 2012; Muhammad *et al.*, 2013) and related species, such as *B. juncea* (Kanchan *et al.*, 2021) which have documented variations in flowering time, maturity, seed yield, oil content, and oil yield.

Furthermore, the analysis revealed significant phenotypic variations across a wide spectrum of agro-morphological traits (Table 2.2). This extensive diversity suggests rich genetic potential within the evaluated germplasm collection, presenting valuable opportunities for targeted crop improvement. Notably, several key traits exhibited particularly wide ranges and substantial mean values, including number of secondary branches (range: 3.15–26.50, mean: 10.89), days to seedling emergence (range: 3.5–18 days, mean: 10.87 days), seed number per silique (range: 3.54–13.22, mean: 7.41), silique length (range: 3.19–8.91 cm, mean: 5.25 cm), 1000-seed weight (range: 2.44–6.05 g, mean: 4.05 g), leaf number (range: 2.40–37.40, mean: 15.21), and leaf length (range: 2.3–11.78 cm, mean: 7.23 cm). This variation in agronomically important traits highlights the potential of this collection as a valuable resource for selecting superior parental lines in breeding programs. For instance, the diversity observed in branching patterns could be exploited to develop cultivars with enhanced canopy architecture, potentially improving light interception and yield. Similarly, the variation in silique characteristics (length and seed number) and seed weight presents opportunities for increasing overall seed yield and quality. The observed phenotypic diversity suggests a wealth of genetic variability within the germplasm that can be leveraged for various breeding objectives. For example, the wide range of days to seedling emergence (3.5–18 days) could be utilized to develop varieties with improved early vigor or adaptability to different planting conditions. The substantial variation in leaf characteristics (number and length) may contribute to the development of cultivars with optimized photosynthetic capacities and stress tolerances. Furthermore, the diversity in 1000-seed weight (2.44–6.05 g) indicates the potential for breeding programs aimed at improving seed size and quality, which are crucial factors for market acceptance and processing efficiency. The considerable variation in silique traits, both in terms of length (3.19–8.91 cm) and seed number per silique (3.54–13.22), presents opportunities for enhancing reproductive efficiency and overall yield potential. Collectively, these findings emphasize the importance of this germplasm collection as a genetic reservoir for *B. carinata* improvement programs. The wide diversity observed across multiple traits provides plant breeders with a robust foundation for developing cultivars tailored to specific agronomic needs, environmental conditions, and market demands. The results align with previous reports on *B. carinata* (Yousuf *et al.,* 2011; Yared *et al.,* 2012; Tesfaye *et al.,* 2014) and other *Brassica* species (Kanchan et al., 2021, Anjali *et al.,* 2022), which have documented extensive variation in morphological and agronomic traits.

In addition, this study revealed significant ($P < 0.05$) variation in physiological traits among *B carinata* accessions as well (Table 2.2), highlighting the rich diversity within the evaluated germplasm. This physiological plasticity presents valuable opportunities for targeted crop improvement and adaptation to diverse environmental conditions. Key findings include: stomatal characteristics (number of stomata: range 153.8–297.8, mean 227.56; stomatal width: range 5.40–60.5 µm, mean 21.39 µm; stomatal conductance: range 118.0–399.7 mmol m^{-2} s^{-1}, mean 283.8 mmol m^{-2} s^{-1}), gas exchange parameters (photosynthesis rate: range 4.62–34.87 µmol m^{-2} s^{-1}, mean 15.52 µmol m^{-2} s^{-1}; transpiration rate: range 2.54–15.4 mmol m^{-2} s^{-1}, mean 7.42 mmol m^{-2} s^{-1}), photosynthetic efficiency: chlorophyll fluorescence (Fv/Fm): range 0.86–0.92, mean 0.89 (Table 2.2). The extensive variation in stomatal characteristics, particularly the number (153.8–297.8) and conductance (118.0–399.7 mmol m^{-2} s^{-1}), suggests significant diversity in water use efficiency and gas exchange capacity among accessions. This variation could be instrumental in developing cultivars adapted to different moisture regimes or with improved drought tolerance. The wide range observed in photosynthesis rates (4.62–34.87 µmol m^{-2} s^{-1}) indicates substantial differences in carbon assimilation capacity among accessions. This diversity presents opportunities for selecting high-performing accessions with enhanced biomass production and potentially higher yield potential. Chlorophyll fluorescence values (Fv/Fm) ranging from 0.86 to 0.92 suggest overall good photosynthetic efficiency across the accessions, with potential for selecting accessions with superior light utilization capacity.

This physiological diversity provides a robust foundation for breeding programs aimed at developing *B. carinata* cultivars with enhanced environmental adaptability and stress tolerance. Breeders can leverage this variation to select accessions exhibiting superior physiological traits such as efficient gas exchange, optimal stomatal function, and increased photosynthetic efficiency. These selections can contribute to the development of cultivars better adapted to challenging environments, thus promoting sustainable agriculture in the face of climate variability. The results align with earlier studies on related Brassica species, including oilseed *Brassicas* (Muhammed and McNeilly, 2004) canola (Roubina and Muhammad, 2006) and cabbage (Albino and Giancarlo, 2005), which all consistently reported substantial variation in similar physiological traits.

2.3.1.3. Accessions with Mean Performance Exceeding the Population Mean

The analysis of 386 *B. carinata* accessions revealed substantial phenotypic diversity, with a significant proportion of accessions (27.9–80.6%) surpasses the population mean values for various morpho-physiological traits (Table 2.2). This extensive variation underscores the rich genetic potential within the evaluated accessions. Key findings include vegetative traits: leaf number: 75.59% (293 accessions) exceeded the mean, leaf width: 51.55% (199 accessions) surpassed the mean, secondary branches: 40.67% (157 accessions) showed above-average values and plant height: 50.26% (194 accessions) were taller than the mean. Among the reproductive traits: silique number: 36.01% (139 accessions) exceeded the mean, seeds per silique: 41.45% (160 accessions) surpassed the average, silique length: 36.27% (140 accessions) were above the mean, 1000-seed weight: 43.78% (169 accessions) showed higher than average values. From yield components such as oil content: 27.98% (108 accessions) exceeded the mean, seed yield: 49.74% (192 accessions) surpassed the average.

Likewise, physiological traits: stomatal number: 77.98% (301 accessions) showed above-average values, photosynthetic rate: 47.15% (182 accessions) exceeded the mean, transpiration rate: 31.09% (120 accessions) surpassed the average, stomatal conductance: 80.57% (311 accessions) were above the mean and chlorophyll fluorescence: 39.12% (151 accessions) showed higher than population average values (Table 2.2). This extensive variation within the germplasm collection highlights its rich morpho-physiological diversity, presenting a valuable resource for breeding programs. The observed diversity allows for the strategic combination of favorable traits in cultivar improvement efforts. For instance, accessions exhibiting superior values for both yield components (e.g., silique number, seeds per silique) and physiological traits (e.g., photosynthetic rate, stomatal conductance) could be selected as potential parents for developing high-yielding, stress-tolerant cultivars. The findings are consistent with previous reports on *B. carinata* (Abebaw *et al.*, 2019) and related species such as *B. juncea* (Anjali *et al.*, 2022) consistently documented high mean values exceeding population averages for traits including plant height, seed yield, and oil content.

Table 2.2: Mean square, range, and mean values of the agro-morphological and physiological traits of 386 *B. carinata* accessions evaluated at HARC (2022–2023)

Traits	Accession (385)	Sub-Block (37)	Replication (1)	Season (1)	R^2 (%)	CV (%)	Range (Min-Max)	Mean + SE	# Accession $\bar{x} > \mu$
DE (day) [a]	18.104***	1.173^{ns}	0.764***	67.34**	89.98	27.68	3.5-18	10.87+0.15	308
PL (cm)	18.104***	1.173 ns	0.764 ***	67.34^{ns}	89.98	27.68	3.5-18	10.87+0.15	128
NL (#)	5.226***	0.004 ns	3.526 ***	1.051^{ns}	93.95	27.15	1.09-10.57	5.95+0.08	293
LL (cm)	55.883***	3.603 ns	1.387***	1.424^{ns}	96.54	24.76	2.40-37.40	15.21+0.27	152
LW (cm)	4.282***	0.003 ns	3.171 ***	0.535^{ns}	98.88	20.25	2.3-11.78	7.23+0.07	199
NPB (#)	2.939***	0.005 ns	3.235 ***	3.538^{ns}	92.86	24.34	1.40-8.75	4.98+0.06	301
NSB (#)	11.644***	0.007 ns	0.112 ***	6.715^{ns}	97.89	23.91	4.75-25.00	10.09+0.12	157
DF (day)	35.416***	0.008 ns	5.963 ***	3.847 **	95.64	18.63	3.15-26.50	10.89+0.21	258
FIL (cm)	251.15***	1.5n49 ns	1.553 ***	7.286^{ns}	99.8	9.57	81.0-140.0	117.07+0.57	128
DH (day)	0.131***	0.015 ns	0.186 ns	0.006^{ns}	90.55	8.56	0.57-1.92	1.38+0.01	220
PH (m)	541.85***	77.698***	68.35***	690.28^{ns}	88.20	5.08	102.5-196	168.94+0.84	194
DS (cm)	0.125***	0.002***	1.82 ***	0.004^{ns}	99.44	2.61	0.72-2.15	1.64+0.01	198
SN (#)	1.866***	0.108 ns	1.244 ***	0.005^{ns}	96.65	7.48	0.89-5.75	3.44+0.05	139

SL (cm)	3569.5***	104.94 ns	747 ns	129.7 ^{ns}	81.99	28.96	15.1-546.1	99.99+2.15	160
SD (mm)	2.406***	0.169 ns	1.689 ***	0.653 ^{ns}	94.54	7.16	3.19-8.91	5.25+0.06	148
NSS (#)	0.346***	0.004 ns	4.01 ns	0.06 ns	97.75	8.65	0.76-3.46	2.36+0.02	194
NSP (#)	9.225***	0.07***	0.07***	0.06 ^{ns}	99.28	27.00	3.54-13.22	7.41+0.11	139
TSW (g)	304527***	429.5 ***	377.41**	4220 ^{ns}	98.17	4.96	70.2-3460	750.9+19.9	169
SY (t/ha)	1.46***	0.003 ns	0.05***	0.10 ^{ns}	87.64	21.07	2.44-6.05	4.05+0.04	192
OC (%)	1101.86**	0.03***	0.03***	0.08 ^{ns}	78.42	14.97	37.88-46.98	43.28+0.12	108
OY (t/ha)	11.10***	0.40 ns	74.07 ns	26.11 ^{ns}	96.79	5.44	2.65-2.69	2.71+0.01	192
NS (#)	3890.86**	16.11***	15.49***	104.4 ^{ns}	94.82	16.24	11.75-12.40	12.08+0.62	301
WS (□m)	1674.8***	698.6 ns	695.2 ns	260.4 ns	71.75	3.02	153.8-297.8	227.56+1.47	168
PR (µmol m-2 s-1)	219.5***	238.3 ns	175.7 ns	126.2 ns	81.03	28.53	5.40-60.5	21.39+0.53	182
TR (mLs-1)	55.19***	5.54***	13.97***	131.9 *	98.99	15.16	4.62-34.87	15.52+0.27	120
SC (mmol m-2 s-1)	9.04***	3.14 ns	2.241***	870.2*	81.24	22.91	2.54-15.4	7.42+0.11	311
CF (FU)	0.015***	0.011^{ns}	1.215 ^{ns}	3.219 ^{ns}	68.65	6.86	0.86-0.92	0.78+0.00	151

[a] refer to table 2.1 for the abbreviations of the traits. The numbers within the braces in the title bar represent the degrees of freedom of the corresponding sources of variation. R^2; coefficient of determination, CV; coefficients of variation, SE; standard error. $\bar{x}$; accessions mean. >; greater than symbol. µ; population mean.*, **, *** and ^{ns} indicates significant at $p < 0.05$, $p < 0.01$, $p < 0.001$ and non- significant at $p > 0.05$ respectively.

2.3.1.4. Comparison of Means of Accessions to Checks

Compared with the checks, the *B. carinata* accessions presented superior performance across several traits, including faster germination; increased vegetative development (evidenced by increased leaf number, branching, stem thickness, and plant height); greater seed yield and oil content/yield; wider stomata; elevated photosynthetic rates; and improved chlorophyll fluorescence (Table 2.3). This extensive phenotypic variation presents breeders with an opportunity to leverage the germplasm collection for targeted selection of parental lines. By combining desired traits, such as high-seed yield and oil-rich accessions with improved plant architecture and maturity characteristics, breeders can develop superior cultivars. These findings are in direct agreement with previous reports (Muhammed and McNeilly, 2004; Tesfaye *et al.,* 2014; Anjali *et al.,* 2022), which documented significant variation in branch number, seed/oil yield, and content among *B. carinata* accessions.

2.3.1.5. Comparison of Means of the Top 5% Accessions with Population and Checks

Analysis of the top 5% (19 accessions) revealed superior mean performance for most traits, with the exception of leaf length and width, stem diameter, 1000-seed weight, number of stomata, and transpiration rate, when compared to both the population mean and check varieties (Table 2.3). Notably, when selecting accessions based on seed yield, oil content, and oil yield, significant increases were observed compared to both the population mean and the check. Specifically accessions: acc-192, acc-194, acc-377, acc-1, acc-386, acc-247, acc-235, acc-294, acc-302, acc-112, acc-331, acc-152, acc-55 and acc-72 demonstrated seed yield increases ranging from 16.70% to 44.30%. Similarly, acc-386, acc-1, acc-21, acc-383, acc-192, acc-31, acc-12, acc-381, acc-193, acc-378, acc-331, acc-235, acc-302, acc-375, acc-72, acc-124, and acc-152 showed oil content increases between 27.4% and 58.7%, while acc-192, acc-194, acc-1, acc-193, acc-386, acc-152, acc-377, acc-331, acc-299, acc-126, acc-281, acc-43, acc-328, acc-72, acc-77 and acc-17 exhibited oil yield increases ranging from 22.6% to 35.8% (Table 2). Furthermore, when selecting accessions based on critical agro-morphological traits such as leaf number, length, and diameter, plant height, days to flowering and harvest, seed yield, oil content, and oil yield, accessions: acc-301, acc-192, acc-1, acc-193, acc-311, acc-386, acc-173, acc-381, acc-331, acc-131, acc-194, and acc-108 exhibited top performance (Table 2.3). This suggests that these Top performing accessions hold significant potential as parents for breeding programs aimed at developing high-yielding, stress-tolerant cultivars tailored for diverse end uses. These Top performing accessions also represent a valuable resource for further characterization and prioritization to create offspring with maximized genetic variation, crucial for successful marker-assisted selection (MAS) and genomic selection (GS) programs. These findings are in line with previous reports on *B. carinata* (Mekonnen *et al.,* 2015) and *B. juncea* (Bhardwaj *et al.*, 2013) which also identified superior accessions.

Table 2.3: Comparison of the means of 21 agro-morphological traits and six physiological traits of the *B. carinata* accessions to checks

Traits	$\bar{x}$ of Accessions	$\bar{x}$ of Checks	Difference	T-value	P-value	High performing accessions (n= 19)
Days to emergence (day)	10.865	8.08	2.785	0.064	0.012	
Petiole Length (cm)	6.909	5.942	0.967	0.831	0.026	
No. Leaves (#)	21.171	15.1	6.071	0.533	0.011	
Leaf Length (cm)	7.216	8.09	-0.87	0.966	0.087	301, 281, 311, 21, 381,51, 331, 271, 131, 351, 291, 371,
Leaf Width (cm)	4.972	5.687	-0.72	0.972	0.175	171, 141, 341, 321, 211, 221, 31
No. Primary Branch (#)	10.074	8.3	1.774	0.986	0.02	
No. Secondary Branch (#)	11.5	8.864	2.636	0.174	0.032	
Days to Flower (day)	108.04	100.6	7.44	0.822	0.001	301, 281, 311, 21, 381, 51, 331, 131, 271, 351, 291,371
Flower Inflorescence (cm)	1.377	1.181	0.196	0.084	0.018	331, 131, 21, 381, 35, 281, 11, 171, 141, 136, 121, 31,
Days to Harvest (day)	165.9	150.8	15.1	0.095	0.001	220, 88, 217, 224, 244, 353, 245, 286, 18, 22, 66, 166
Plant Height (m)	2.142	1.639	0.503	0.124	0.017	21, 381, 51, 131, 331, 271, 311, 281, 171, 291, 371,351
Diameter of Stem (cm)	3.939	3.902	0.037	0.662	0.051	261, 271, 68, 264, 262, 3, 96, 1, 91, 134, 147, 115, 140

Silique No (#)	108.3	106.75	1.55	0.720	0.011	239, 21, 311, 51, 331, 271, 301, 131, 281, 351, 291,371
Silique Length (cm)	5.238	6.328	-1.09	0.804	0.003	5, 13, 27, 10, 33, 21, 46, 193, 167, 173, 171, 250, 282
Silique Diameter (mm)	2.354	2.508	-0.15	0.578	0.438	126, 162, 362, 138, 244, 237, 319, 133, 128, 206, 213,8
No. Seed/ Silique (#)	7.403	7.65	-0.25	0.996	0.059	111, 61, 1, 81, 101, 215, 106, 136, 54, 145, 224,107,234
No. Seed/ Plant (#)	936	928.5	7.5	0.970	0.002	239, 21, 311, 51, 331, 271, 301, 131, 281, 351, 291, 371
1000-Seeds Weight (g)	4.056	4.064	-0.01	0.964	0.391	369, 45, 23, 35, 60, 292, 204, 322, 13, 305, 38, 49, 5,35
Seed Yield (t/ha)	2.738	2.652	0.086	0.807	0.038	192, 194,377,193,1,386,247,235,294, 302, 112,331,152
Oil Content (%)	46.835	46.822	0.013	0.156	0.023	386,1,21,383,31,12,381,193,378,331,235,302,375,72,12
Oil Yield (t/ha)	13.252	12.955	0.297	0.816	0.030	192,194,1,193,386,152,377,331,299,126,281,43,328,72
No. Stomata (#)	237.34	244.6	-7.26	0.109	0.099	301,281,311,21,381,51,331,271,131,351,291,371,171
Width of Stomata (µm)	27.335	25.48	1.86	0.115	0.002	68, 288, 93, 214, 311, 287, 67, 97, 94, 286, 285, 325, 95
Photosynthesis Rate (µmol m^{-2} s^{-1})	15.527	15.22	0.31	0.204	0.053	192, 124,377,112,1,386,55,331,235,302,375,72,171, 98
Transpiration Rate (mLs^{-1})	7.405	8.152	-0.75	0.693	0.545	120, 231, 112, 241, 42, 46, 345, 44,190,225, 183,25,188
Stomatal Conductance (mmol m^{-2} s^{-1})	342.5	336.03	6.47	0.825	0.013	311, 281, 301, 291, 386, 321,192,171,31,331, 351,21,51
Chlorophyll Fluorescence (FU)	0.785	0.757	0.03	0.509	0.003	112,124,181,331,55,231,101,251,221,71,384,261,191

$\bar{x}$; accessions mean. >; greater than symbol. µ; population mean

2.3.2. Analysis of Variance Components

Genetic variability is crucial for successful crop improvement (Sankar *et al.*, 2006). This study assessed the variability among *B. carinata* accessions using genotypic variance (σ^2G), phenotypic variance (σ^2Ph), genotypic coefficient of variation (GCV), and phenotypic coefficient of variation (PCV) (Table 2.4). Thousand seed weight (TSW) exhibited the highest genetic (304088.36) and phenotypic (304527.0) variance, indicating substantial potential for the selection and improvement of this trait. Conversely, chlorophyll fluorescence (CF) showed minimal variance ($\sigma^2G = 0.01$, $\sigma^2Ph = 0.02$), suggesting limited scope for improvement through direct selection alone. Significant genetic variance was also observed in stomatal number (SN), silique length (SL), stomatal width (SW), photosynthesis rate (PR), oil content, and plant height, all of which offer breeders a greater opportunity to select superior accessions for future breeding programs.

Genotypic (GCV) and phenotypic (PCV) coefficients of variation were estimated for various agro-morphological traits of *B. carinata*. GCV ranged from 7.33% for oil yield to 1194.28% for oil content ha^{-1}, while PCV ranged from 2.79% (transpiration rate) to 1224.43% (oil content ha^{-1}). According to Deshmukh *et al.* (1986), GCV and PCV values below 10% were classified as low, 10-20% as medium, and above 20% as high. Leaf width, flower inflorescence length, plant height, stomatal width, and chlorophyll fluorescence exhibited medium GCV and PCV. Oil yield showed a low GCV, and oil yield and transpiration rate showed a low PCV. However, most traits displayed high GCV and PCV, with PCV consistently exceeding GCV. This signifies the existence of substantial genotypic and phenotypic variability in *B. carinata* suggests a significant potential for improving seed and oil yields through selection. The higher

PCV values relative to GCV values for these traits suggest that environmental influence was minimal, and genotypic effects largely determined the phenotypic expression. Thus, phenotypic selection of these traits would be effective. These findings are consistent with those of previous studies that reported high GCV and PCV values for key agro-morphological traits in *B. carinata* (Tesfaye *et al.,* 2014; Amsalu, 2019b).

2.3.3. Heritability and Genetic Advance

The genotypic coefficient of variation (GCV) alone does not fully capture heritable variation. Thus, broad-sense heritability (H^2) was estimated to assess its potential for selection. In the present study, H^2 ranged from 31.74% for leaf width to 99.86% for thousand-seed weight (Table 2.4). According to Singh (2001), H^2 values above 80% are considered high, 60-79% moderately high, 40-59% medium, and below 40% low. In this study, all traits except leaf width exhibited high H^2 values, indicates a relatively minor environmental influence on phenotypic expression and suggests that selection based on phenotypic performance would be effective due to the likely dominance of additive gene effects. Thus, the incorporation of these traits into future breeding programs holds considerable promise for improvement. These high H^2 estimates are consistent with those of previous studies on *B. carinata* (Abebe *et al.,* 2009; Amsalu, 2019b)*, B. rapa* (Jan *et al.,* 2017), and *B. napus* (Ilyas *et al.,* 2018), in which high H^2 values have been reported for most morpho-agronomic traits.

Heritability alone is insufficient to predict the selection response. Integrating heritability and genetic advances has been reported to provide a more realistic prediction of potential breeding success (Rahim *et al.,* 2011). Therefore, the genetic advance (GA) was then estimated. GA values, represent the expected improvement in the next generation following selection, ranged widely (Table 2.4), from 0.11 (chlorophyll fluorescence) to 715.55 (number of seeds per plant). Significant GA was observed for several traits including 715.55 for number of seeds per plant, 119.49 for stomatal conductance per plant, 75.33 for number of siliques per plant, 58.01 for stomatal number per plant, 22.87 t ha^{-1} for oil yield, 4.61% for oil content per plant, and 0.53 t ha^{-1} for seed yield. These results suggested that the selection of superior accessions could lead to substantial gains in these traits. For instance, selecting the top 5% high-yielding parents could theoretically result in offspring with mean values increased by the aforementioned GA values. The results are in agreement with the findings of Rahim *et al.* (2011) in *B. napus*.

In addition, combining heritability estimates with genetic advance expressed as a percentage of the mean (GAM) reported provides a more comprehensive assessment of selection potential (Deepthi *et al.,* 2013; Allard, 1960). According to Johnson *et al.* (1955), GAM was categorized as high (>20%), moderate (10-20%), and low (<10%). In the present study, most traits exhibited high GAM (Table 2.4), except for silique diameter (14.27%), seed yield (13.14%), chlorophyll fluorescence (13.72%), leaf length (18.30%), and leaf width (10.96%), which showed moderate GA. A low GAM was also observed for flower inflorescence length (0.43%), plant height (0.30%), silique length (2.22%), thousand-seed weight (0.23%), and stomatal width (8.98%). Traits with both high heritability and GAM are likely controlled by additive gene action and are therefore highly responsive to selection (Panes and Sukhatme, 1995). The observed high heritability and GAM for most traits indicate a strong additive genetic component, suggesting that selection in subsequent generations based on these traits would be highly effective. Therefore, traits such as days to flowering, length of flower inflorescence, number of siliques, seeds per silique, siliques per plant, stomatal number, stomatal conductance, and oil content were expected to respond favorably in the next generation to selection. These findings are consistent with those of previous studies on *B. carinata* (Tesfaye *et al.*, 2014) and *B. juncea* (Sangeeta *et al.*, 2019).

Table 2.4**:** Estimates of variance components, heritability and genetic advance for 27 morpho-physiological traits of *B. carinata* accessions

Trait	δ2g	δ2ph	GCV	PCV	H^2	GA	GAM
DE [a]	17.65	18.10	38.65	39.14	97.50	6.04	55.60
PL	17.05	18.10	37.99	39.14	94.19	3.14	28.85
NL	4.36	5.23	35.09	38.42	83.43	9.10	152.68
LL	51.60	55.88	47.23	49.15	92.34	2.78	18.30
LW	1.36	4.282	16.13	28.62	31.74	0.79	10.96
NPB	2.67	2.94	32.79	34.42	90.75	4.51	90.58
NSB	10.88	11.64	32.70	33.82	93.47	8.10	80.30
DF	34.86	35.42	54.22	54.65	98.43	22.73	208.72
LFI,	238.00	251.15	13.18	13.54	94.77	0.50	0.43
DH	0.12	0.13	25.07	26.23	91.45	31.01	2247.002
PH	529.28	541.85	13.62	13.77	97.68	0.50	0.30
SD	0.12	0.13	21.34	21.59	98.32	1.96	119.30
NS	1.62	1.87	36.95	39.71	86.55	75.33	2189.76
SL	3504.22	3569.5	59.20	59.75	98.17	2.22	2.22
DS	2.11	2.41	27.64	29.54	87.49	0.75	14.27
NSS	0.34	0.35	24.79	24.92	99.08	4.40	185.76
NSP	8.21	9.23	38.68	40.99	89.02	715.55	9656.48

Trait	δ2g	δ2ph	GCV	PCV	H^2	GA	GAM
TSW	304088.36	304527.0	73.44	73.49	99.86	1.76	0.23
SY	1.45	1.46	29.68	29.83	99.04	0.53	13.14
OC	1047.49	1101.86	1194.28	1224.43	95.08	4.61	170.25
OY	10.06	11.10	7.33	7.70	90.61	22.87	52.84
SN	3786.03	3890.86	509.36	516.36	97.31	58.01	480.22
SW	1600.12	1674.8	17.58	17.98	95.54	20.43	8.98
PR	202.66	219.5	66.56	69.27	92.33	9.99	46.71
TR	49.75	55.19	45.45	2.79	90.15	3.95	25.45
SC	7.40	9.04	36.67	40.53	81.83	119.49	1610.41
CF	0.01	0.02	12.18	15.64	60.00	0.11	13.72

[a] refer to table 2.1 for the abbreviations of the traits. δ^2g = Genotypic variance, δ^2ph = Phenotypic variance, GCV: Genotypic coefficient of variation, PCV: Phenotypic coefficient of variation, H^2: Broad sense heritability, GA: Genetic advance, GAM: Genetic advance as percent of the mean.

2.3.4. Genotypic and Phenotypic Correlation of Traits

Yield is a complex trait resulting from the interaction of various characteristics that contribute to positive or negative associations (Burhan, 2007). Correlation studies provide reliable information regarding the nature and direction of selection for combining high yield potential with desirable traits (Srivastava *et al.,* 2018). To evaluate the magnitude of the association between various traits and economic yields of *B. carinata*, genotypic and phenotypic correlation coefficients were computed (Table 2.5). The analysis revealed positive and significant correlations ($P \leq 0.05$) between seed yield, oil yield, and oil content with various agro-morphological and physiological traits suggests that selecting for one trait could indirectly influence others. In addition, a few traits showed negative and significant ($P \leq 0.05$) correlations, whereas others showed either positive or negative, statistically non-significant ($P > 0.05$) correlations. Genotypic correlation coefficients exceeded the corresponding phenotypic correlation coefficients for most characteristics, indicating strong inherent associations among the studied traits. Lower phenotypic correlation coefficients suggest reduced phenotypic expression due to environmental influences. These findings are consistent with those of previous studies on *B. carinata* (Abebe *et al.,* 2009; Yared, 2011) and soybeans (Malek *et al.*, 2014).

Analysis of genotypic and phenotypic correlations revealed significant associations between seed yield (SY) and various morpho-physiological traits in *B. carinata*. Although SY exhibited highly significant positive correlations with most phenological, yield-related, and physiological parameters, it showed non-significant negative correlations with stomatal number (rg = -0.12, rp = -0.15) and width (rg = -0.07, rp = -0.07) (Table 2.5). Oil content was significantly positively correlated with leaf number (rg = 0.76, rp = 0.63), length (rg = 0.53, rp = 0.44), width (rg = 0.69, rp = 0.61), seeds per plant (rp = 0.58), 1000-seed weight (rp = 0.38), SY (rp = 0.74), oil yield (rg = 0.87, rp = 0.83), and photosynthesis rate (rg = 0.74, rp = 0.62); however, negative correlations were observed with stomatal number (rg = -0.21, rp = -0.15), and chlorophyll fluorescence (rg = -0.19, rp = -0.12). Similarly, oil yield exhibited strong positive correlations with most agro-morphological and physiological traits but negative correlations with petiole length (rg = -0.18, rp = -0.19) and chlorophyll fluorescence (rg = -0.22, rp = -0.13). Leaf number, length, and width showed highly significant positive correlations with most morpho-physiological traits, excluding days to emergence and silique width. Primary and secondary branch numbers were positively correlated with most traits, except silique length and diameter, which showed non-significant correlations. Plant height, days to flowering, and days to harvest exhibited significant positive correlations with the majority of the agronomic and physiological traits. These findings suggest that indirect selection for improved SY, oil yield, and oil content could effectively enhance multiple morpho-physiological traits, potentially accelerating breeding progress compared with direct selection alone. This is consistent with previous studies on *B. carinata* (Yared, 2011; Fekadu, 2021), *canola* (Khan *et al.*, 2008), and *B. juncea* (Singh et al., 2018), which have reported significant positive correlations between seed yield, oil yield, oil content, and several agro-morphological and physiological traits.

Table 2.5**:** Genotypic (below diagonal) and phenotypic (above diagonal) correlation coefficients of 27 quantitative traits of 386 *B. carinata* accessions tested at HARC during 2022 and 2023

Trait	DSE	PL	NL	LL	LW	NPB	NSB	DF	LFI	DH	PH	SD	NS	SL
DE[a]		-0.09	-0.07	-0.08	-0.09	-0.07	-0.08	0.82***	-0.06	0.91***	-0.12	-0.07	-0.13	0.03
PL	-0.11		-0.38*	0.99***	0.69**	0.27*	0.38*	-0.08	-0.51*	-0.09	0.79**	0.16	0.84***	0.10
NL	-0.09	0.98***		0.67**	-0.57*	0.99***	0.99***	-0.10	0.55*	-0.10	0.72**	0.17	0.82***	0.80***
LL	-0.15	1.00***	0.78**		0.99***	0.97***	0.96***	-0.08	0.62**	-0.10	0.81***	0.16	0.82***	0.12
LW	-0.12	0.72**	0.69**	1.00***		0.98***	0.97***	-0.08	-0.66**	-0.09	0.80***	0.15	0.82***	0.11
NPB	-0.08	0.38*	0.99***	1.00***	0.99***		0.98***	-0.11	-0.57*	-0.10	0.73**	0.16	0.81***	0.09
NSB	-0.09	0.49*	1.00***	0.99***	0.99***	1.00***		-0.10	0.55*	-0.10	0.72**	-0.18*	0.83***	0.08
DF	0.91***	-0.09	-0.13	-0.12	-0.14	-0.18	-0.18		-0.04	-0.48*	-0.04	-0.15	-0.08	-0.07
LFI	-0.08	0.59*	0.62**	0.76**	0.75**	0.64*	0.56*	-0.08		-0.08	0.79**	0.11	-0.47*	0.17
DH	0.95***	-0.12	-0.11	-0.15	-0.12	-0.14	-0.13	0.57*	-0.11		-0.08	-0.09	-0.09	-0.09
PH	-0.13	0.87***	0.84***	0.85***	0.86***	0.79**	0.75**	-0.07	0.82***	-0.13		0.15	0.63*	0.12
SD	-0.09	0.17	0.19	0.68**	0.64**	0.23*	0.22	-0.19	0.14	-0.11	0.18		0.15	-0.22*
NS	-0.16	0.85***	0.89***	0.92***	0.89***	0.86***	0.85***	-0.09	0.52*	-0.13	0.67**	0.19		0.04
SL	0.05	0.12	0.84***	0.61**	0.58**	0.15	0.12	-0.12	-0.24*	-0.16	0.17	0.37*	0.09	
DS	-0.18	0.16	0.16	-0.25*	0.17	0.16	0.13	0.15	-0.03	-0.07	0.09	-0.08	0.16	-0.08
NSS	-0.07	0.24	0.97***	0.74**	0.85***	0.69**	0.77**	-0.19	0.3	-0.17	-0.28*	0.07	0.19	0.19
NSP	-0.10	0.73**	0.71**	0.73**	0.75**	0.68**	0.72**	-0.17	0.48*	-0.19	0.67**	0.23	0.82***	0.23
TSW	0.06	-0.25	0.49*	0.68**	0.72**	-0.19	-0.19*	0.14	-0.25	-0.07	-0.41*	-0.18	-0.24	-0.13
SY	0.08	0.15	0.94***	0.86***	0.95***	0.96***	0.97***	0.06	0.08	0.08	0.14	0.08	0.74**	0.77***
OC	0.03	0.14	0.76**	0.53**	0.69**	0.17	0.15	-0.15	-0.04	-0.06	0.04	0.09	0.17	0.09
OY	0.09	-0.18*	0.88***	0.78**	0.87***	-0.19*	0.23	-0.02	0.09	0.05	0.16	0.04	0.18	0.08
SN	-0.11	0.38*	0.42*	0.66**	0.77**	0.51*	0.38*	-0.15	-0.27*	-0.14	-0.22*	-0.07	-0.29*	0.16
SW	-0.17	-0.05	-0.05	0.06	0.19	-0.03	-0.07	0.08	-0.13	0.04	-0.15	-0.14	-0.12	-0.07
PR	0.13	-0.08	0.84***	0.79**	0.88***	-0.09	-0.11	0.09	-0.04	0.07	-0.06	-0.07	-0.06	-0.18*
TR	0.21	0.09	0.79**	0.68**	0.79**	0.07	0.08	0.16	0.07	0.08	0.08	-0.05	0.15	0.06
SC	-0.18	0.86***	0.86***	0.86***	0.87***	0.86***	0.88***	-0.18*	-0.58*	-0.19	0.71**	0.41*	0.82***	-0.32*
CF	-0.04	0.62**	0.67**	0.71**	0.58*	0.71**	0.62**	-0.07	0.31	-0.09	0.53*	0.17	0.54*	-0.16

Trait	DS	NSS	NSP	TSW	SY	OC	OY	SN	SW	PR	TR	SC	CF
DE^{a}	-0.15*	-0.04	-0.10	0.03	0.04	0.01	0.05	-0.04	-0.06	0.07	0.03	-0.16*	-0.01
PL	0.11	-0.19*	-0.20*	-0.21*	0.11	0.11	-0.15*	-0.34*	-0.04	-0.06	0.06	0.33*	0.21
NL	0.53*	0.62***	0.76**	0.81***	0.86***	0.63**	0.81***	0.95***	-0.03	0.98***	0.75**	0.80***	0.58*
LL	0.12	0.70**	0.69**	0.72**	0.81***	0.44*	0.84***	0.73**	-0.03	0.87***	0.71**	0.83***	0.21
LW	0.11	0.67**	0.72**	0.91***	0.87***	0.61**	0.95***	0.94	-0.03	0.91***	0.86***	0.83***	-0.12
NPB	0.10	0.55*	0.67**	0.17	0.92***	0.13	0.86***	0.75**	-0.01	0.58*	0.45*	0.40*	0.35*
NSB	0.10	0.13	0.76**	0.19	0.95***	0.17	0.97***	0.63**	-0.03	0.65**	0.48*	0.52*	-0.28*
DF	0.05	-0.14	-0.12	0.07	0.02	-0.07	-0.00	-0.08	0.05	0.07	0.10	-0.15	-0.05
LFI	-0.01	0.92***	0.88***	-0.20*	0.06	-0.01	0.06	0.21	-0.09	-0.01	0.04	0.53*	-0.27*
DH	-0.04	-0.13	-0.15	-0.05	0.04	-0.04	0.03	-0.08	0.01	0.05	0.05	-0.12	-0.07
PH	0.06	0.83***	0.96***	-0.20*	0.87***	0.00	0.88***	0.79**	-0.07	0.93***	0.84***	0.64**	0.34*
SD	-0.03	0.72**	0.92***	-0.15	0.60**	0.04	0.61	-0.02	-0.05	-0.04	-0.01	0.38*	0.10
NS	0.09	0.12	0.78**	-0.17	0.68**	0.10	0.14	0.25	-0.07	-0.02	0.11	0.70**	-0.48*
SL	-0.06	0.86***	0.99***	-0.08	0.70***	0.06	0.91***	0.11	-0.01	0.11	0.01	0.20	-0.12
DS		-0.14	-0.03	0.98***	0.95***	0.08	0.70*	0.02	0.11	0.47*	-0.08	0.13	-0.09
NSS	-0.18		0.92***	0.72**	0.90***	0.03	0.89***	0.77**	0.01	0.86***	0.35*	0.80***	0.05
NSP	-0.29*	0.97***		0.78**	0.86***	0.58*	0.94***	0.80****	-0.04	0.92***	0.41*	0.86***	-0.39*
TSW	0.99***	0.79**	0.84***		0.90***	0.38*	0.85***	-0.04	0.03	0.86***	0.66**	0.35*	0.05
SY	0.98***	0.99***	0.98***	0.96***		0.74**	0.94***	-0.03	-0.04	0.92***	0.35*	0.18	0.20
OC	-0.24*	0.60**	0.64**	0.76*	0.82***		0.83***	-0.15*	-0.07	0.62**	-0.02	0.09	-0.12*
OY	0.83***	0.91***	0.97***	0.92***	0.98***	0.87***		0.01	-0.05	-0.50*	0.03	0.12	-0.13*
SN	0.08	0.79**	0.85***	-0.07	-0.12	-0.21*	0.04		-0.01	-0.08	0.83***	0.76**	0.15
SW	0.21	0.07	-0.09	0.08	-0.07	-0.14	-0.08	-0.06		0.00	0.88***	0.56*	-0.06
PR	0.59*	0.88***	0.97***	0.92***	0.95***	0.74**	0.68**	-0.21*	0.05		0.06	-0.06	0.01
TR	-0.17	0.42*	0.59*	0.75**	0.42*	-0.08	0.09	0.88***	0.91***	0.12		0.06	0.05
SC	0.29	0.84***	0.88***	0.47*	0.25	0.13	0.19	0.81***	0.61*	-0.08	0.17		0.46*
CF	-0.17	0.18	0.44*	0.13	0.37*	-0.19*	-0.22*	0.18	-0.15	0.06	0.09	0.54*	

[a] refer to table 2.1 for the abbreviations of the traits.*, ** and *** indicates significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$ respectively

2.3.5. Multivariate Principal Component and Cluster Analysis

2.3.5.1. Principal Component Analysis

Principal component analysis (PCA) offers a dimensionality reduction technique for high-dimensional data (Venujayakanth et al., 2017). It transforms complex datasets into a smaller set of key principal components (PCs) that capture the most significant variation and underlying patterns within the original data (Chahal and Gosal, 2006). Eigenvalue analysis guides the selection of informative PCs to retain for further analysis (Bhardwaj *et al.,* 2013). Factor loadings associated with a particular PC quantify the influence of individual traits on that PC (Muhammed and McNeill, 2004). Traits with higher absolute factor loading values (closer to 1 or -1) exert a stronger influence on the PC (Sharma, 2006). Additionally, the sign of the factor loading (+/-) reflects whether a trait is positively or negatively correlated with the PC (Anjali *et al.,* 2022). This information empowers breeders to prioritize traits on the basis of their contribution to PCs and develop targeted breeding strategies for efficient crop improvement.

PCA of *B. carinata* accessions revealed distinct patterns of variation (Figure 2.2). The top five PCs, each with eigenvalues exceeding 1.341, collectively explained 61% of the total variance (Table 2.4). These findings deviate slightly from previous reports (Kumar *et al.*, 2021; Li *et al.,* 2022) on *B. carinata*, where the top five PCs explained 68.2% and 70.8% of the total variation, respectively. This variation likely arises from differences in the experimental environments, accessions employed, and traits included in each study.

PCA revealed that the first PC (PC1) explained 35.59% of the variation observed among the *B. carinata* accessions (Table 2.6). Traits with high factor loadings exerted the strongest influence on PC1, include petiole length: 0.982, leaf width: 0.981, leaf length: 0.978, number of primary and secondary branches: 0.965, stomatal conductance: 0.871, silique number: 0.858, number of seeds per plant: 0.764, plant height: 0.808, flower inflorescence length: 0.658, chlorophyll fluorescence: 0.564 and stomatal number: 0.348. These findings highlight the critical role these traits play in morpho-physiological variation within the *B. carinata* germplasm. Focusing on these traits during breeding programs has the potential to increase selection efficiency for targeted improvement. These results align with previous study on *B. juncea* (Kumar *et al.*, 2021) that PC1 explained 37.8% of the total variation, with petiole length, leaf width, and leaf length identified as the top contributing factors.

The second PC (PC2) accounted for 7.9% of the observed variation and grouped accessions primarily on the basis of seed yield (factor loading: 0.770) and oil yield (factor loading: 0.799) (Table 2.6). This pattern suggests that prioritizing these traits during breeding would be beneficial for developing cultivars with superior seed and oil yields. These findings are consistent with previous reports on *B. carinata* (Li *et al.,* 2022; Peng *et al.,* 2023) highlighted the importance of PC2 in explaining variation and its strong association with oil and seed yield traits.

The remaining PCs (PCs 3–5) explained additional variation and grouped accessions on the basis of specific trait combinations (Table 2.4). PC3 accounted for 6.82% of the variation and primarily reflected growth and development traits, with days to emergence (0.518), flowering (0.651), and harvest (0.591) exhibiting positive factor loadings, whereas oil content displayed a negative factor loading (-0.311). PC4 explained 5.91% of the variation and grouped accessions on the basis of silique characteristics (number: 0.654, width: -0.531, and length: 0.433) and photosynthetic efficiency (0.439). Finally, PC5 explained 4.97% of the variation, with 1000-seeds weight (-0.619), stem diameter (0.385), and transpiration rate (-0.311) contributed most significantly. By identifying these key traits associated with each PC group, breeders can strategically select parental lines during hybridization, allowing them to target desired traits for cultivar improvement. These findings are consistent with previous studies on *B. carinata* (Abebaw *et al.*, 2019; Dejen *et al.*, 2016), which reported associations between specific PCs and distinct sets of traits related to growth, yield, oil content, and other physiological processes.

Table 2.6: PCA and Eigen value of the agro-morphological and physiological traits of 386 *B. carinata* accessions

Traits	PC1	PC2	PC3	PC4	PC5
Days to emergence (day)	-0.124	0.449	0.518	0.263	0.089
Petiole Length (cm)	0.982	0.027	0.081	-0.067	-0.004
No. Leaves (#)	0.965	0.042	0.076	-0.132	-0.049
Leaf Length (cm)	0.978	0.020	0.081	-0.059	0.014
Leaf Width (cm)	0.981	0.024	0.079	-0.061	0.002
No. Primary Branch (#)	0.965	0.042	0.064	-0.122	-0.032
No. Secondary Branch (#)	0.965	0.045	0.068	-0.130	-0.044
Days to Flower (day)	-0.134	0.438	0.651	0.055	0.112
Flower Inflorescence (cm)	0.658	-0.054	0.088	0.164	0.159
Days to Harvest (day)	-0.141	0.453	0.591	0.099	0.217
Plant Height (m)	0.808	-0.046	0.109	0.087	0.106

Traits	PC1	PC2	PC3	PC4	PC5
Silique No (#)	0.858	0.035	0.034	-0.044	-0.104
Diameter of Stem (cm)	0.217	-0.149	-0.211	0.166	0.385
Silique Length (cm)	0.132	-0.129	-0.141	0.433	0.398
Silique Diameter (mm)	0.089	-0.066	0.008	-0.531	0.359
No. Seed/ Silique (#)	0.265	-0.262	-0.122	0.654	-0.124
No. Seed/ Plant (#)	0.764	-0.116	-0.049	0.366	-0.183
1000-Seeds Weight (g)	-0.242	0.070	0.063	-0.135	-0.619
Seed Yield (t/ha)	0.137	0.770	-0.484	0.004	0.098
Oil Content (%)	0.131	0.250	-0.311	-0.036	-0.072
Oil Yield (t/ha)	0.174	0.799	-0.551	-0.013	0.071
No. Stomata (#)	0.348	-0.072	0.026	-0.185	-0.019
Width of Stomata (µm)	-0.057	-0.082	0.058	-0.191	0.067
Photosynthesis Rate (µmol m^{-2} s^{-1})	-0.060	0.220	-0.050	0.439	-0.166
Transpiration Rate (mLs^{-1})	0.071	0.137	0.112	0.230	-0.311
Stomatal Conductance (mmol m^{-2} s^{-1})	0.871	-0.087	-0.067	0.083	0.181
Chlorophyll Fluorescence (FU)	0.564	0.141	0.032	-0.117	-0.414
Eigenvalue	9.610	2.144	1.842	1.596	1.341
Individual (%)	35.59	7.94	6.82	5.91	4.97
Cumulative (%)	35.59	43.53	50.36	56.26	61.23

PC1, PC2, PC3, and PC4 represents principal components 1, 2, 3, and 4, respectively

2.3.5.2. Relationship of traits and principal components

The PCA biplot visualization in Figure 2.2 depicts strong correlations between the analyzed morpho-physiological traits in relation to the first two PCs, PC1 and PC2. Traits such as leaf size (petiole length, leaf width, and leaf length), branching (number of primary and secondary branches), stomatal conductance, silique number, plant height, number of seeds per plant, flower inflorescence length, and chlorophyll fluorescence all presented high positive loadings on PC1. This pattern suggests that these traits are positively correlated and tend to co-vary. Similarly, seed yield, oil content, and days to maturity presented the highest positive loadings on PC2, indicating a strong correlation between them and their tendency to vary together. Understanding these inter-trait relationships can inform selection strategies to avoid unintentionally impacting correlated traits during the selection process. These findings corroborate previous reports (Dejen *et al.,* 2016) on *B. carinata*, where traits with high loadings on different principal components were reported to indicate strong correlations and linked patterns of variability.

The PCA biplot (Figure 2.2) offers further insights on the basis of trait vector direction and length. Traits with vectors pointing in the same direction exhibit positive correlations. Notably, the co-aligned vectors for petiole length and primary and secondary branch numbers all share positive correlations, indicating that they tend to increase or decrease together. Similarly, the same aligned seed yield and oil content vectors were positively correlated. Vector length, on the other hand, reflects a trait's contribution to a particular PC. Longer vectors, such as the oil yield for PC2 and branch number for PC1, indicate a stronger influence on their respective principal components. Selection strategies can leverage these findings by targeting accessions with positive correlations for desired traits while considering the impact of highly influential traits (represented by long vectors) on overall variation. These observations are consistent with previous reports (Dejen *et al.*, 2016; Abebaw *et al.*, 2019) that documented co-aligned trait vectors for leaf size, branching, seed yield, and oil content in *B. carinata*.

Overall, this study revealed that petiole length (PL: 0.982), leaf width (LW: 0.981), leaf length (LL: 0.978), the number of primary and secondary branches (NPB = NSB = 0.965), stomatal conductance (SC: 0.871), oil yield (OY: 0.799), and seed yield (SY: 0.770) were the most significant contributors to the phenotypic diversity observed among *B. carinata* accessions. Therefore, accessions exhibited superior mean values for these traits represent valuable targets for selection breeding.

The PCA biplot (Figure 2.2) again visualizes the extent of genetic variation among *B. carinata* accessions. The accessions positioned closer together and to the origin presented similar quantitative trait values, suggesting minimal genetic divergence. Conversely, accessions located far from the origin and each other represent more genetically divergent accessions. Notably, accessions 1, 2, 15, 38, 58, 64, 69, 74, 80, 151, 152, 184, 192, 194, 204, 267, 301, 307, 311, 331, and 381 presented greater genetic divergence, making them promising candidates for crop improvement. These accessions can be directly selected for breeding programs or utilized as diverse parental lines in hybridization strategies to introduce valuable genetic variation and enhance breeding outcomes.

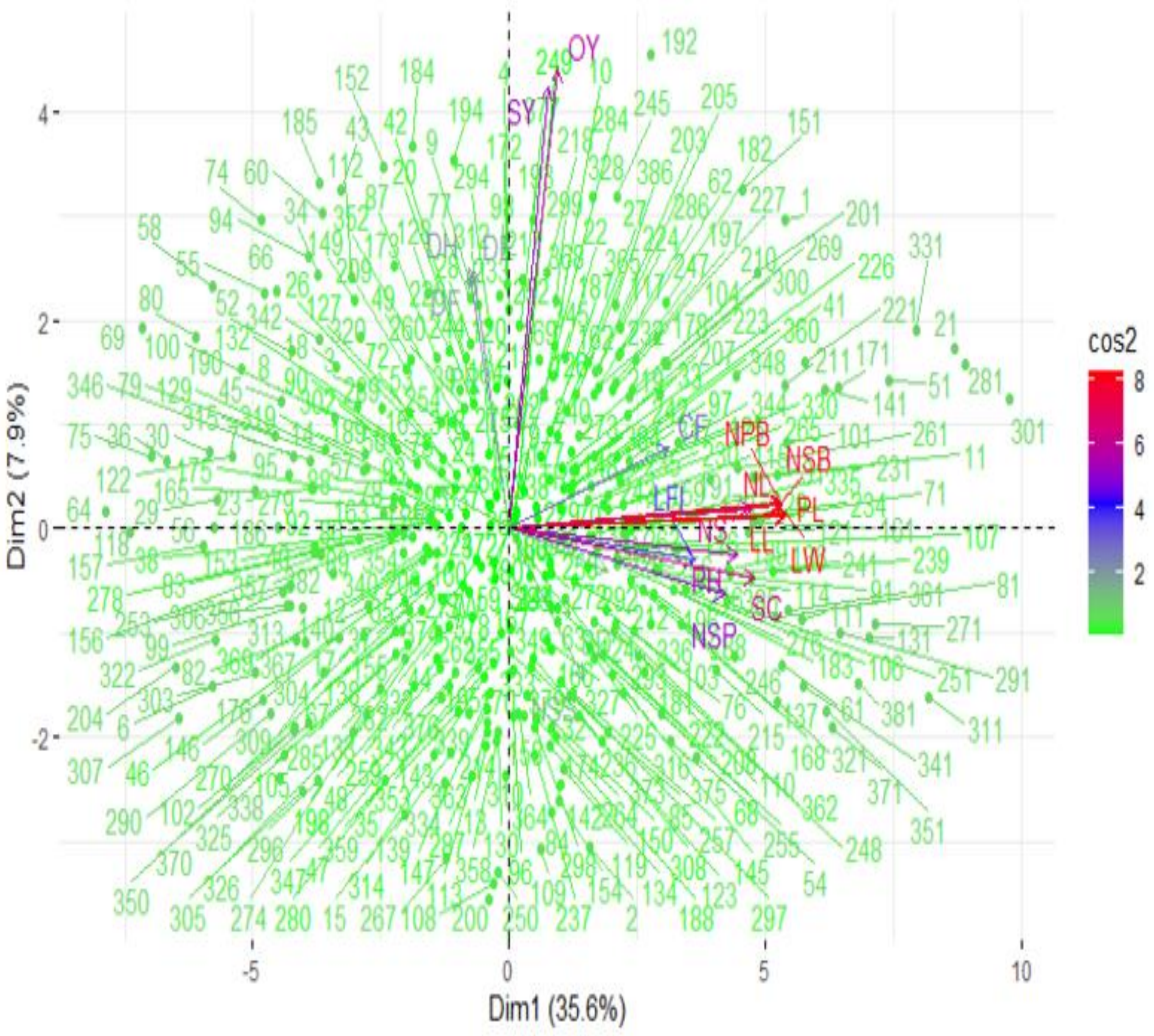

Figure-2.2 Biplot of key agro-morphological and physiological traits of 386 *B. carinata* accessions. The color code and scale in the figure legend illustrate the trait-principal component relationships in this study. Red/Pink (values ~8) indicate key traits strongly correlated with principal components, while Purple/Blue (values 4-6) represents important traits with varying correlation levels compared to red/pink. Green (values ≤ 2) denotes individual *B. carinata* accessions

2.3.5.3. Cluster Analysis

To classify the 386 *B. carinata* accessions on the basis of genetic similarity, cluster analysis was performed via the UPGMA algorithm. UPGMA prioritizes minimizing variation within clusters, making it ideal for identifying groups with high intra-cluster similarity and low inter-cluster similarity (Muhammed and McNeilly, 2004). Euclidean distance matrices were used to quantify genetic dissimilarity for cluster analysis.

Cluster analysis based on 27 morpho-physiological traits revealed four distinct diversity clusters among the 386 *B. carinata* accessions (Figure 2.3). Cluster 1 (C1) comprised 153 accessions (39.64%) from various Ethiopian regions, while Cluster 2 (C2) was the largest with 157 accessions (40.67%) with Oromia (79), Amhara (49), Tigray (8), Benishangul-Gumuz (5), South Ethiopia (5), commercial varieties (4), Southwest Ethiopia (4), Central Ethiopia (2), and Harari (1) (Table 2.7). Clusters C3 and C4 consisted of 12 and 64 accessions, respectively, representing almost all the *B. carinata* growing regions (Figure 2.3, Table 2.7). This distribution of accessions suggests that genetic diversity is independent of geographic origin and likely reflects inter-regional germplasm exchange, common ancestral origin, or both. The presence of accessions from the same region within different clusters highlights morpho-physiological diversity, probably arising from genetic variation due to ancestral differences or recombination events during hybridization. These findings align with those of previous studies on *B. carinata* (Hunde *et al.,* 2031) and sorghum (Beyene *et al.,* 2013) which stated that genetic factors play a more significant role in determining diversity than geographical location of the crop germplasm.

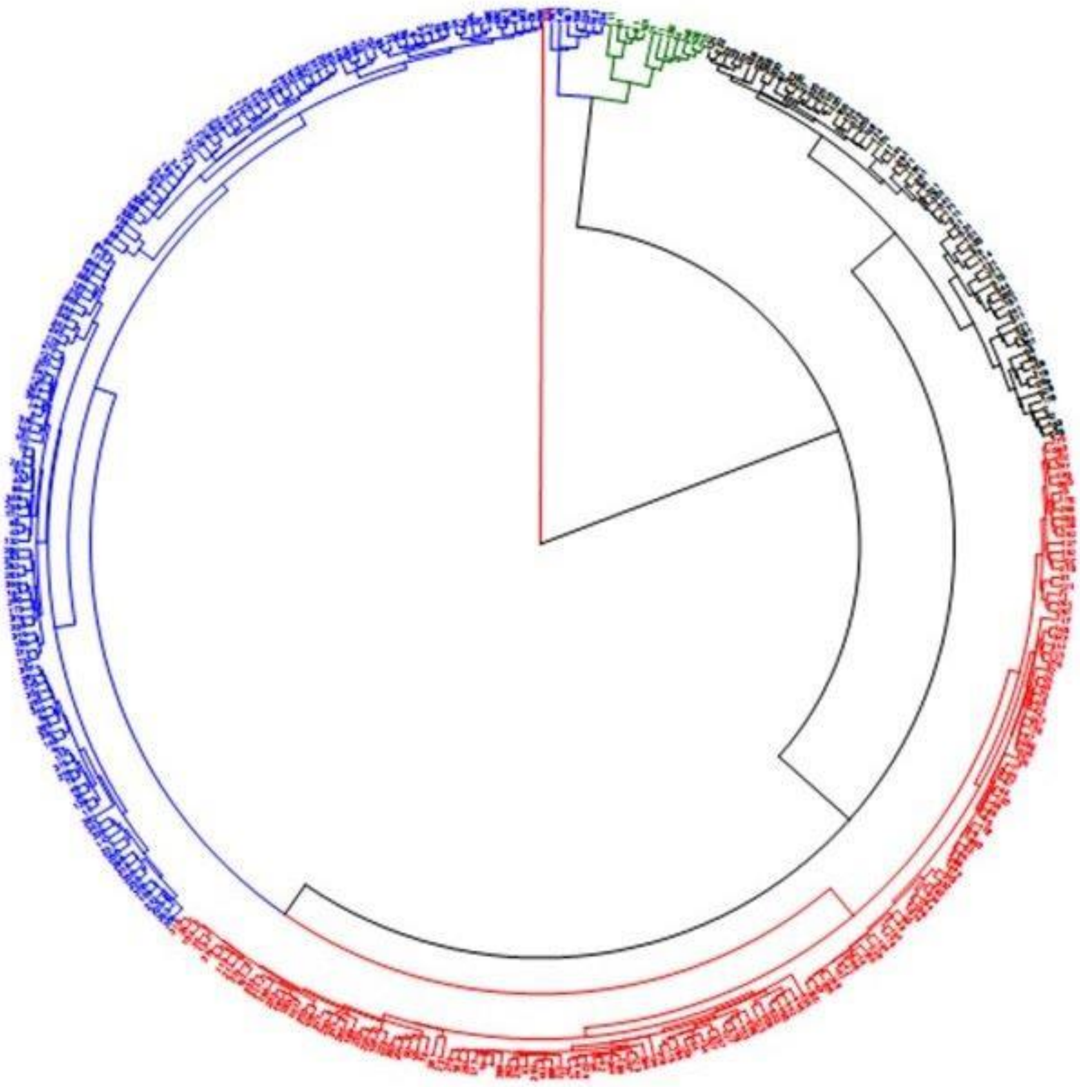

Figure-2.3 Dendrogram of the 386 *B. carinata* accessions and their genetic relationships. The color codes indicate the accession clusters: blue (Cluster 1), red (Cluster 2), green (Cluster 3), and black (Cluster 4).

Table 2.7**:** Cluster assignment of 386 *B. carinata* accessions from various collection regions.

Collection region	Cluster				Total number of accessions/region
	C1	C2	C3	C4	
Amhara	46	49	9	16	120
Benishangul Gumuz	7	5	0	2	14
Central Ethiopia	1	2	1	1	5
Harari	2	1	0	1	4
Oromia	78	79	2	39	198
South Ethiopia	8	5	0	1	14
South West Ethiopia	5	4	0	0	9
Tigray	5	8	0	4	17
Commercial	1	4	0	0	5
Total number of accessions/cluster	153	157	12	64	386

The abbreviations C1, C2, C3, and C4 represents clusters 1, 2, 3, and 4, respectively.

2.3.5.4. Estimation of Intra-and Inter-Cluster Distance

Cluster analysis revealed significant genetic distances (8.84–18.21) among the *B. carinata* accessions (Table 2.8). The greatest divergence was observed between clusters C3–C4 (18.21) and C2–C4 (14.44), suggesting substantial morpho-physiological trait variation. This diversity indicates considerable potential for exploiting heterosis through breeding crosses between these genetically distinct clusters. Conversely, smaller genetic distances between clusters C1–C2 (8.84) suggest limited variation and potentially lower success rates for intra-group crosses. These results are consistent with those of previous reports on *B. carinata* (Yewale *et al.*, 2016) and *B. juncea* (Weerakoon and Somaratne, 2010) which highlight the value of utilizing accessions with high genetic distances in breeding programs to maximize heterosis.

Further analysis of intra-cluster diversity revealed that C2 exhibited the greatest intra-cluster distance (6.71) and mean genetic distance (5.23) (Table 2.8). This pattern suggests a more diverse genetic composition within C2, implying that breeders can strategically select diverse parental lines from this cluster to broaden the genetic basis of the cultivars. These findings are consistent with previous reports on *B. carinata* (Bhardwaj *et al.,* 2013) and sesame (Geleta *et al.,* 2020) in which clusters with varying levels of intra-cluster diversity have been reported.

Table 2.8: Intra- (bold diagonal) and inter-cluster (off-diagonal) distances, along with mean distances and cluster sizes, in *B. carinata* cluster analysis on the basis of 21 agro-morphological and six physiological traits

Cluster	C1	C2	C3	C4	Mean distance
C1	**6.542**				4. 915
C2	8.844	**6.714**			5.234
C3	14.265	9.592	**5.974**		2.821
C4	12.997	14.443	18.213	**6.396**	3.746

The abbreviations C1, C2, C3, and C4 represents clusters 1, 2, 3, and 4, respectively.

2.3.5.5. Analysis of Cluster Means

Cluster mean analysis revealed distinct agro-morphological and physiological trait profiles within the *B. carinata* germplasm (Table 2.9). The accessions in C1 displayed earlier flowering times (114.61 days) with high stomatal density (233.33) and conductance (348.02 mmol m^{-2} s^{-1}). These traits may be favorable for water management strategies. In contrast, C2 accessions exhibited rapid emergence (10.19 days) and earlier maturity (159.69 days) alongside superior vegetative growth, as evidenced by longer petioles (8.07 cm) and leaves (9.34 cm), wider leaves (6.5 cm), taller plants (1.84 m), thicker stems (3.8 cm), and longer inflorescences (1.56 m). Additionally, C2 accessions presented greater numbers of leaves (22.31), primary branches (13.05), secondary branches (16.58), and siliques (188.85) and exhibited superior yield-related traits, including more seeds per plant (2002.23), greater seed yield (1.75 t/ha), increased oil content (44.88%), and greater oil yield (0.78 t/ha). Furthermore, the C2 accessions also showed increased photosynthetic activity, with the highest rates of photosynthesis (18.73 μmol m^{-2} s^{-1}), transpiration (7.83 mL s^{-1}), and chlorophyll fluorescence (0.89 FU). These findings suggest that C2 accessions represent a valuable resource for breeding strategies. Breeders can select these accessions on the basis of targeted combinations of traits to improve seed and oil yield, adapt to specific environments, and improve photosynthetic efficiency.

Conversely, C3 and C4 presented lower values for most traits but exhibited the highest values for 1000-seed weight (4.30 g), silique length (5.36 cm), and silique diameter (2.38 mm) (Table 2.9). These findings suggest their potential utility in breeding programs that specifically target improvements in these traits. These results are consistent with previous *B. carinata* studies on germination, maturity, and vegetative growth (Mekonnen *et al.*, 2015); early flowering and stomatal traits (Dejen *et al.*, 2016); and yield and oil content (Peng *et al.*, 2023), all of which reported significant variation in trait distribution among clusters.

Table 2.9: Cluster means of 386 *B. carinata* accessions based on 21 agro-morphological and six physiological traits

Traits	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Days to emergence (day)	10.895	10.192	11.086	10.744
Petiole Length (cm)	7.633	8.067	4.572	6.219
No. Leaves (#)	20.99	22.308	11.136	15.589
Leaf Length (cm)	8.719	9.037	5.954	7.501
Leaf Width (cm)	6.232	6.496	3.927	5.202
No. Primary Branch (#)	12.66	13.050	8.147	10.378
No. Secondary Branch (#)	15.557	16.581	7.594	11.216
Days to Flower (day)	114.612	117.231	119.216	116.340
Flower Inflorescence (cm)	1.502	1.563	1.221	1.445
Days to Harvest (day)	168.037	159.692	171.392	168.076
Plant Height (m)	1.804	1.864	1.455	1.699
Diameter of Stem (cm)	3.547	3.838	3.337	3.458
Silique No (#)	137.201	188.850	70.237	101.955
Silique Length (cm)	5.34	5.305	5.060	5.364
Silique Diameter (mm)	2.335	2.123	2.359	2.380
No. Seed/ Silique (#)	9.094	11.534	5.745	7.730
No. Seed/ Plant (#)	1194.226	2002.226	397.039	759.387
1000-Seeds Weight (g)	3.877	4.002	4.303	3.925
Seed Yield (t/ha)	1.744	1.754	1.718	1.706

Traits	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Oil Content (%)	43.464	44.876	43.130	43.213
Oil Yield (t/ha)	75.755	78.829	74.163	76.081
No. Stomata (#)	236.332	233.778	218.736	230.558
Width of Stomata (µm)	21.774	19.073	21.770	21.116
Photosynthesis Rate (µmol m^{-2} s^{-1})	14.887	18.728	15.456	15.580
Transpiration Rate (mLs^{-1})	7.892	7.826	7.226	7.344
Stomatal Conductance (mmol m^{-2} s^{-1})	348.023	373.856	222.309	299.890
Chlorophyll Fluorescence (FU)	0.858	0.889	0.756	0.772

FU- Fluorescent unit

2.4. Conclusion and Prospects

This study revealed significant phenotypic variation across 27 agro-morphological and physiological traits of Ethiopian mustard accessions. Diversity was evident through a wide range of trait means, principal component analysis (PCA), and cluster analysis. Notably, 19 Top performing accessions demonstrated substantial increases in seed yield (16.70% to 44.30%) and oil content (27.4 % to 58.7%) compared with both the population mean and checks. PCA identified five principal components explaining over 61% of the variation, with leaf size, branching, seed yield, and oil yield contributing the most significantly. Cluster analysis revealed four groups with substantial genetic distances (8.84–18.21) between accessions, with the highest divergence occurring between C3 and C4. C2 exhibited the highest intra-cluster distance (6.71) and mean genetic distance (5.23), suggesting valuable morpho-physiological variation in breeding programs.

From this study, two distinct groups of promising accessions are proposed for *B. carinata* improvement based on their unique traits and potential applications.

- Group-1 (acc-192, acc-386, acc-1, acc-235, acc-294, acc-302, acc-112, acc-331, acc-152, acc-55, and acc-72) demonstrated significant potential to enhance seed yield, oil yield, and quality traits.
- Group-2 (acc-192, acc-124, acc-377, acc-112, acc-1, acc-386, acc-55, acc-331, acc-235, acc-302, acc-375, acc-72, acc-171, acc-98, and acc-20) exhibited a distinct profile, demonstrating particular suitability for water management strategies.

In conclusion, the identification of these two distinct groups highlights the rich genetic diversity within *B. carinata* and underscores the potential for targeted breeding efforts to enhance its agronomic performance. Further evaluation across multiple locations is recommended to assess commercial suitability. Future research on seedoil composition and the molecular mechanisms underpinning these traits will be crucial for optimizing breeding strategies and realizing the full potential of these promising accessions.

CHAPTER 3

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3. Variability in Seed Oil Content and Fatty Acid Composition of Ethiopian Mustard (*Brassica carinata* A. Braun) Germplasm

Abstract

Ethiopian mustard (Brassica carinata A. Braun) is a versatile oilseed crop with potential applications in food, biofuel, and industrial sectors. However, its potential has not been fully exploited through breeding because of the limited understanding of genetic variation in oil-related traits. The present study characterized the genetic diversity of 386 B. carinata accessions to identify superior accessions based on their oil content and fatty acid composition. The experiment employed an augmented block design with two replicates. Oil content and fatty acid profiles were determined using nuclear magnetic resonance spectroscopy (NMRS) and near-infrared reflectance spectroscopy (NIRS), respectively. Significant ($p \leq 0.05$*) variation was observed across all traits, with seed oil content ranging from 37.88% to 46.98%. High heritability (85–94%) and genetic advance (22.30–59.29%) were estimated for all traits. Cluster analysis revealed seven distinct groups with significant intercluster distances. Generally, acc-386 for oil content, acc-02 for erucic acid, acc-386 for α-linolenic acid, acc-385 for eicosenoic acid, and acc-309 for stearic acid were identified as promising candidates for industrial applications because of their high oil content and fatty acid levels. Acc-372 for linoleic, acc-326 for oleic, and acc-270 for palmitic acids showed considerable potential for further improvement for edible oil. This study provides valuable insights for future breeding programs, highlighting the utilization of genetic diversity to optimize fatty acid profiles for various end uses. In particular, the identified accessions with high erucic acid contents have the potential to develop sustainable biofuel feedstock from B. carinata.*

Keywords: Ethiopian Mustard; Fatty Acids; Genetic Gain; Genotypic Variation; Heritability; NIRS; NMRS; Oil Content

3.1. Introduction

Ethiopian mustard (*Brassica carinata* A. Braun) is a versatile oilseed crop with significant potential across various sectors (Wikipedia, 2024). It is native to Ethiopia and belongs to the *Brassicaceae* family (Fekadu, 2021). The production of *B. carinata* oil involves pressing and distillation methods, yielding products with distinct characteristics tailored for diverse applications (Transparency Market Research, 2024). The balanced composition of unique monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), and saturated fatty acids (SFAs) has attracted global interest and has gained prominent market value (Ghosh & Bauri, 2018; Rahiel *et al.*, 2020). Despite limited empirical data from Ethiopia, global market analyses have demonstrated significant growth, particularly in Asia-Pacific and North America, with a market value reaching $34,164.9 million in 2023 (Chemanalyst, 2024).

Traditionally utilized for food, lighting and medicinal purposes in Ethiopia (Rahiel *et al.*, 2024), *B. carinata* oil is emerging as a functional food ingredient rich in MUFAs (Research Pulse, 2024). However, its high erucic acid content (31–46%), which exceeds safe consumption limits (< 2% in the EU), has prompted breeders to focus on developing cultivars with lower erucic acid levels for edible purposes while maintaining their value for industrial applications (Ghosh & Bauri, 2018; Ajay *et al.*, 2019). The industrial applications of *B. carinata* are diverse, including biofuel production, feedstock for oleochemical industries, slip agents in plastic films, lubricants, and detergents (Zanetti *et al.*, 2012; Schulmeister *et al.*, 2019). Additionally, the seed meal and hulls of the crop are utilized in animal feed, biofertilizers, and bioenergy production, enhancing its sustainability and economic viability (Mekuria *et al.*, 2018; Walelign *et al.*, 2022; Tesfaye *et al.*, 2023).

The oil yield and quality of *B. carinata* are strongly influenced by seed variety and growing region, which are intricately linked to its genetic architecture (Knowledge Sourcing, 2022). This genetic architecture is complex, governed by multiple genes (quantitative trait loci), and is shaped by environmental factors (Kumar et al. 2022). Studies have shown that both additive and non-additive gene effects control oil content (Schulmeister *et al.,* 2019), and genetic mapping plays a crucial role in elucidating the composition of the oil (Chaudhary *et al.,* 2021). The intricate genetic architecture and multipurpose nature of *B. carinata* drive breeders to conduct extensive genetic variability studies within the available gene pool to fully understand and exploit Top performing accessions for further breeding efforts. Ethiopia has significant potential in this regard, with over 400 *B. carinata* germplasms maintained in the Ethiopian Biodiversity Institute (EBI) (http://www.ebi.gov.et) and the Ethiopian Institute of Agricultural Research (EIAR) (http://www.eiar.gov.et) gene banks. These germplasms represent a rich source of genetic diversity, which is crucial for *B. carinata* trait improvement. A thorough understanding of this genetic diversity is of paramount importance for breeding programs aimed at developing new cultivars with enhanced oil quality traits (El Bassam *et al.,* 2011). In line with this, national *B. carinata* breeding initiatives are primarily focused on manipulating oil composition through interspecific gene transfer, often directed towards industrial applications (http://www.eiar.gov.et). A major breeding objective remain is to increase the content of oleic and linoleic acids while simultaneously reducing the α-linolenic acid content, implying the need for continued exploration of innovative breeding strategies (http://www.eiar.gov.et/holetta/).

Despite its significant potential as a multipurpose oilseed crop and rich germplasm resources, efforts to optimize *B. carinata* through breeding have been impeded by a limited understanding of the genetic variation underlying seed oil content and fatty acid composition. Previous studies have predominantly focused on agronomic traits using limited germplasm collection (Yared *et al.,* 2011; Mekonnen *et al.,* 2014; Fekadu, 2021; Tesfaye *et al.,* 2023; Rahiel *et al.,* 2024), neglecting the crucial aspects of genetic analysis of oil-related traits. This knowledge gap presents a substantial obstacle to fully exploiting the crop's potential and developing improved cultivars with enhanced oil content and optimized fatty acid compositions tailored to specific industrial requirements. Addressing this research gap is crucial for advancing breeding programs and realizing the full agronomic and economic potential of this oilseed crop. To address this, the present study undertook a comprehensive genetic variability analysis of 386 *B. carinata* germplasms based on seed oil content and fatty acid composition. The primary objective was to identify Top performing accessions with superior oil characteristics, which will provide valuable insights for Ethiopian mustard breeding, and highlight the potential of harnessing genetic diversity to improve oil content and fatty acid profiles.

3.2. Materials and Methods

3.2.1. Description of Experimental Site, Plant Material and Eperimental Procedure

A field experiment was conducted at HARC, Holeta, Ethiopia, using 386 *B. carinata* accessions. The experiment employed an Augmented Block design with two replications in accordance with the established national guidelines for *B. carinata* cultivation. Refer to Chapter 2 for detailed descriptions of the experimental site, plant material, and experimental procedures, including a site map of the accessions collection points (Figure 2.1) and a complete list of accessions (Appendix Table 2.1).

3.2.2. Data Collection

For sample seed collection, ten mature plants from the middle rows of each accession plot were randomly selected. After meticulous cleaning to remove foreign material, the seeds of each accession were oven-dried (D-6450 Hanau, Heraeus Instruments, Germany) at 78°C for 2.5 hours to achieve a uniform moisture content of 11% for oil content and fatty acids determination as follows.

(a) Seed Oil content (%): According to the International Organization for Standardization (ISO 660:2009), a 22 g seed sample from each accession was oven-dried (78°C, 2.5 h), cooled, and the oil content (%) was determined using nuclear magnetic resonance spectroscopy (NMRS) equipment (Newport 4000, Newport Instruments Ltd., UK), following established protocols from the Oregon State University Seed Laboratory (https://seedlab.oregonstate.edu/).

(b) Fatty Acid Composition (%): Three grams of cleaned seeds from each accession was separately dried overnight at 60°C. The oil's erucic, linoleic, α-linolenic, oleic, eicosenoic, palmitic, and stearic acid contents were analyzed using near-infrared reflectance spectroscopy (NIRS) equipment (Win Scan version 1.5, 2000, Intrasoft International, L.L.C, Luxembourg) as described by Shenk and Westerhaus (1993). The fatty acid content was expressed as the percentage of each individual fatty acid relative to the total fatty acid content of the sample. To ensure data reliability, three NMRS and NIRS measurements were obtained for each sample and averaged for the analysis.

3.2.3. Statistical Analysis

Prior to conducting the analysis of variance (ANOVA), the data were checked for adherence to ANOVA assumptions using SAS 9.4 (SAS Institute, 2023). As the yearly ANOVA showed that year had no significant effect ($p > 0.05$) on on any oil or fatty acid traits, data from both years were combined for subsequent analyses.using the same package at a significance level of $\alpha = 0.05$. To address potential data scale discrepancies, the combined means were standardized (mean = 0, variance = 1) using R 4.3.2 (R Development Core Team, 2023).

To dissect the sources of phenotypic variation within the accessions, variance component analysis was done in R (R Development Core Team, 2023) utilizing the variability package (Popat et al., 2020). For a comprehensive description of the specific variance component analysis procedures applied, please refer to the data analysis section in Chapter 2. The unweighted pair group method with arithmetic mean (UPGMA) for agglomerative hierarchical clustering with Euclidean distances, as described by Lance & Williams (1967), was employed using the FactoMineR package in R (Le *et al.*, 2024) to group accessions based on similarities. The optimal number of clusters was determined using Pseudo F and Pseudo T statistics, calculated via the FactoMineR package. Genetic distances between and among accessions were computed from Euclidean distances, as outlined by Gan *et al.* (2007), using the Factoextra package in R (Kassambara, 2022) to quantify the genetic divergence between individual accessions or groups within the study.

3.3. Results and Discussion

3.3.1. Accessions Variations Based on Univariate Statistics for Oil and Fatty Acid Traits

Analysis of variance (ANOVA) results revealed highly significant ($p \leq 0.001$) differences in the oil content and fatty acid composition among the 386 evaluated *B. carinata* accessions (Table 3.1). This substantial genetic diversity within the germplasm collection indicates its promising potential as a valuable resource for breeding programs aimed at enhancing oil content and tailoring fatty acid profiles to meet specific market needs. These findings align with those of previous studies on *B.* carinata (Yared *et al.,* 2010; Yousef *et al.*, 2015; Fekadu, 2021), which has consistently reported significant genetic variability in these traits.

Implementation of blocking and replication effectively minimized for field-related variability, as evidenced by the non-significant ($p > 0.05$) impact of these factors on oil content and fatty acid composition (Table 3.1). This confirms the effectiveness of these experimental practices in controlling extraneous variation and isolating genetic influences on the traits of interest. Comparable results have been reported in studies on *B. juncea* (Kanchan *et al.,* 2021) indicated that the effectiveness of blocking and replication in minimizing field variability reinforces the crucial role of robust experimental design in generating reliable and accurate research findings.

The present study revealed a strong relationship between the studied traits and *B. carinata* performance, as evidenced by the consistently high coefficients of determination (R^2), ranging from 81.48 – 99.24% (Table 1). R^2 quantifies the proportion of variation in the dependent variable explained by the independent variable(s) with values ranging from 0 to 100% (Prey et al. 2020). High R^2 values (> 80%) signify robust predictive capacity, facilitating informed selection decisions in breeding programs (Turney, 2022). In this study, erucic and eicosenoic acids exhibited exceptionally high R^2 values of 99.24% and 96.89%, respectively, accounting for nearly all the observed variation among accessions. These results strongly support the use of these traits as primary selection criteria for breeding strategies. Comparatively, stearic acid displayed a somewhat lower R^2 of 81.48 %, it still demonstrated a significant positive correlation with *B. carinata* performance, suggesting its potential utility as a selection criterion for specific breeding goals. Low R^2 values can be attributed to high data variability, inadequate experimental design, inherently nonlinear relationships among variables, measurement errors, and limited ranges of independent variables (Turney, 2022). Careful experimental planning and appropriate statistical modeling can help improve the explanatory power of that particular trait/variable. These results corroborate the findings of Montgomery (2005) regarding the predictive power of elevated R^2 values approaching 100%.

Table 3.1 further illustrates that the experimental design effectively minimized extraneous variation, as reflected by consistently low coefficient of variation (CV) values (2.5 –8.97%) below 30% for all traits. CV measures relative variability across experimental treatments or studies used to assess experimental precision (Saleh, 2024). In field experiments, lower CV values (<30%) indicated higher precision and reliability, whereas higher values (>30%) suggested greater variability and potential experimental design issues (Kozak *et al.*, 2013). The exceptionally low CV for linoleic acid (2.5%) suggests that uncontrolled factors have a minimal influence on trait variability, allowing for meaningful comparisons across future *B. carinata* field experiments. Such comparisons could be used to assess experimental precision and inform the optimization of experimental designs for research investigating phenotypic or genetic diversity. These findings align with those of Gomez and Gomez (1984), who reported that CV values below 30% were acceptable in agricultural field experiments. Collectively, the high R^2 and low CV values in this study provide compelling evidence for a strong positive relationship between the analyzed traits and *B. carinata* performance, with minimal influence from uncontrolled environmental factors.

Table 3.1: Mean squares for oil content and fatty acid traits in 386 *B. carinata* accessions at HARC, 2022-2023

Traits	Accession (380)	Checks (4)	Block (Rep=37)	Replication (1)	R^2 (%)	CV (%)
Oil content (%)	5.510^{***}	10.654^{***}	629.640^{ns}	1477.087^{ns}	93.84	5.44
Erucic acid (%)	15.505^{***}	45.670^{***}	28.765^{ns}	45.797^{ns}	99.24	8.13
Linoleic acid (%)	4.109^{***}	10.504^{***}	1.726^{ns}	0.135^{ns}	89.98	2.5
α-linolenic acid (%)	8.806^{***}	31.328^{***}	7.631^{ns}	0.012^{ns}	98.82	2.63
Oleic acid (%)	0.845^{***}	0.276^{***}	2.009^{ns}	0.572^{ns}	91.26	6.41
Eicosenoic acid (%)	0.822^{***}	0.427^{***}	1.705^{ns}	0.212^{ns}	96.89	3.08
Palmitic acid (%)	0.355^{***}	0.372^{***}	0.279^{ns}	0.003^{ns}	87.72	7.40
Stearic acid (%)	0.200^{***}	0.159^{***}	0.252^{ns}	0.101^{ns}	81.48	8.97

Numbers in brackets in the title bar indicate degrees of freedom (df) associated with each source of variation. R^2; coefficient of determination, CV; coefficients of variation. *** and ^{ns} indicates significant at $p < 0.001$ and non- significant at $p > 0.05$ respectively

3.3.2. Variation in Mean and Range of Accessions for Oil Content and Fatty Acid Profiles

Analysis of the 386 *B. carinata* accessions revealed significant ($p < 0.05$) variations in oil content and fatty acid profiles, as illustrated in Figure 3.1. The oil content ranged from 37.88% to 46.98%, with a mean of 43.28%. Among the fatty acids, erucic acid displayed the widest variation (34.05%–56.57%), with a mean of 49.0%. Similar notable variations were observed for linoleic acid (10.75–19.99%, mean: 16.21%), α-linolenic acid (7.47–22.40%, mean: 13.91%), oleic acid (4.79–12.16%, mean: 10.99%), eicosenoic acid (3.43–10.09%, mean: 8.17%), palmitic acid (1.33–4.48%, mean: 3.42%), and stearic acid (0.91–2.90%, mean: 1.54%) (Fig. 3.1). These highlights substantial genetic diversity within the *B. carinata* germplasm collection, providing a valuable resource for plant breeding programs seeking to develop cultivars with tailored traits. Accessions with high mean contents of oil, erucic, α-linolenic, ecosenoic, and stearic acids can be selected for breeding cultivars suitable for industrial applications, such as biofuels, lubricants, surfactants, plastics, and cosmetics. Furthermore, accessions with elevated levels of linoleic, oleic, and palmitic acids may be promising to edible oil or nutritionally fortified food products. These results are consistent with previous reports by Ghosh and Bauri (2018) and Fekadu (2021), who reported even higher mean and range variations among the *B. carinata* accessions evaluated.

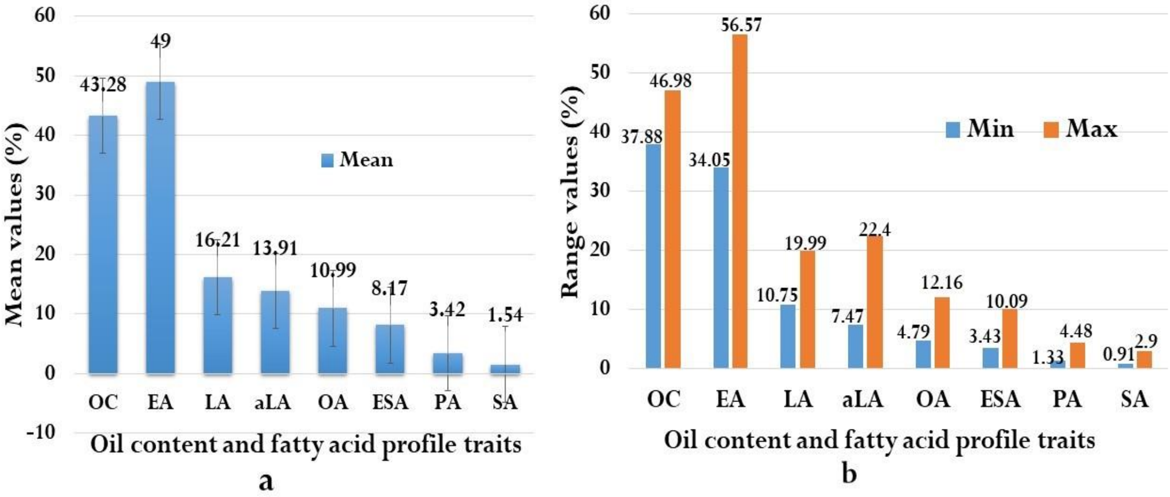

Figure-3.1 Mean + SE (a) and range (b) values of oil content and fatty acids in 386 *B. carinata* accessions

Abbreviations: OC; Oil content, EA; Erucic acid, LA; Linoleic acid, αLA; α-linolenic acid, OA; Oleic acid, ESA; Eicosenoic acid, PA; Palmitic acid, SA; Stearic acid, and SE; Standared Error

3.3.3. Comparison of the Means of Accessions with Check Varieties

Compared to the check varieties, the *B. carinata* accessions exhibited significantly higher mean values for various fatty acids and oil content (Table 3.2). Notably, accessions showed increases in oil content (5.48%), erucic acid (12.74%), α-linolenic acid (12.74%), linoleic acid (30.3%), palmitic acid (48.82%), and stearic acid (48.87%) compared to the checks. These findings underscore the substantial variation existed in oil content and fatty acid composition within the *B. carinata* germplasm collection. These rich diversity provides breeders with a vast pool of accessions from which cultivars enriched with specific fatty acids can be selected and developed for various applications. These results were consistent with those reported by Genet et al. (2005), Shiv et al. (2013), and Yousef et al. (2015), who also observed higher mean values for certain fatty acids and oil contents in accessions than in checks.

3.3.4. Comparison of Means of the Top 5% of Accessions with Population and Checks

The top 5% (n = 19) of *B. carinata* accessions exhibited significantly higher mean values for oil content and several key fatty acids compared to both the overall population and the check varieties (Table 2). Among the Top performing accessions, acc-386 for oil content (43.66-47%), acc-102 for erucic acid (52.4-56.57%), acc-386 for α-linolenic acid (16.3–22.4%), acc-385 for eicosenoic acid (7.8–10.09%), and acc-309 for stearic acid (1.01–2.12%) performed better than the population and check means (Table 3.2). These results indicate significant genetic variation within the *B. carinata* germplasm collection, highlighting the potential for breeding cultivars tailored for industrial applications such as biofuels, lubricants, surfactants, plastics, and cosmetics. Furthermore, acc-372 for linoleic acid (14.8-19.9%), acc-326 for oleic acid (5.02–12.32%), and acc-309 for palmitic acid (2.7-4.48%) were elevated compared to the population and check means (Table 2), suggesting the potential to develop cultivars for edible oil production or nutritionally fortified food products. In general, the identified Top performing accessions represent a valuable resource for further characterization and prioritization to create offspring with maximized genetic variation, which is essential for successful marker-assisted selection (MAS) and genomic selection (GS) programs.

Table 3. 2: Mean comparison of *B. carinata* accessions to checks for oil related traits and the high performing top 5% accessions

Traits	Means of Accession	Means of Checks	Difference	T-value	P-value	High performing accessions (n= 19)
Oil content (%)	43.29	41.04	2.25	1.042	0.012	386,1,21,383,31,12,381,193,378,331,235,302,375,72, 12, 375, 374, 373, 372, 371, 370, 369
Erucic acid (%)	49.04	43.50	5.54	1.481	0.001	102, 332, 321, 261, 30, 76, 278, 114, 146, 91, 279, 213, 216, 171, 175, 274, 15, 55, 67
Linoleic acid (%)	16.21	12.44	3.77	0.426	0.064	372, 307, 173, 60, 313, 53, 126, 193, 46, 68, 301, 360, 34, 232, 154, 332, 328, 39, 36
α-linolenic acid (%)	15.66	13.89	1.77	0.134	0.091	386, 146, 147, 57, 60, 197, 372, 193, 27, 329, 307, 88, 313, 68, 105, 340, 39, 339, 320
Oleic acid (%)	10.89	11.26	-0.37	0.288	0.126	326, 256, 272, 103, 317, 368, 299, 56, 26, 149, 306, 111, 244, 255, 346, 81, 172, 67, 122
Eicosenoic acid (%)	8.178	5.57	2.608	1.261	0.01	385, 352, 351, 35, 5, 363, 46, 156, 2, 279, 229, 163, 265, 276, 90, 280, 12, 212, 160

Palmitic acid (%)	3.419	2.36	1.059	0.102	0.02	270, 40, 98, 384, 21, 243, 173, 320, 101, 63, 181, 18, 203, 301, 86, 296, 102, 202, 328
Stearic acid (%)	1.84	1.24	0.60	0.087	0.073	309, 71, 67, 158, 94, 204, 237, 316, 132, 334, 59, 266, 167, 96, 55, 139, 282, 72, 199

3.3.5. Analysis of Genetic Parameters

3.3.5.1. Genotypic Coefficient of Variation

This study evaluated the genetic variation among *B. carinata* accessions for eight traits using the genotypic coefficient of variation (GCV). According to Deshmukh *et al.* (2012), GCV measures the extent of heritable variation among accessions, with values categorized as low (<10%), moderate (10-20%), or high (>20%). In this study, oil content, erucic acid, and oleic acid exhibited GCV values ranging from 5.44% to 8.45% (Table 3.3), which are considered low and significantly below the previously reported 10–20% GCV for oil content in *B. napus* (Redda *et al.*, 2022).

These results suggest that these traits might be under high selection pressure or have reached a genetic plateau in the studied population. This limited genetic variability could pose challenges for breeders aiming to improve these traits using conventional selection methods.

According to Khan *et al.* (2008), employing innovative breeding strategies, such as marker-assisted selection (MAS), genetic engineering, multi-trait selection, and interspecific hybridization, could be more effective in addressing the challenges posed by low GCV traits.

Conversely, eicosenoic, linoleic, and palmitic acids showed moderate GCVs (11.14–17.26%), indicating a reasonable level of genetic diversity, offering opportunities for incremental improvements through selective breeding. indicating a potential for genetic gain. Notably, α-linolenic and stearic acids demonstrated high GCVs (20.41 and 27.91%, respectively), suggesting substantial genetic diversity with promising candidates that can be exploited for targeted breeding. These results align with those of Mnzava *et al.* (2007) and Fekadu (2021), who reported low, medium, and high GCV estimates for the oil content and most fatty acid traits in Brassica species. While GCV provides valuable insights into genetic variability (Burton & DeVane, 1953), integrating this information with heritability estimates and genetic correlations between traits would offer a more comprehensive understanding of breeding potential (Johnson *et al.*, 1955).

3.3.5.2. Heritability and Genetic Gain

This study assessed broad-sense heritability (H^2) and genetic advance as a percentage of the mean (GAM) for eight traits in *B. carinata* to evaluate their potential for genetic improvement. According to Burton and DeVane (1953), H^2 quantifies the proportion of phenotypic variation attributable to genetic factors, and is categorized as high (>60%), moderate (30–60%), or low (0–30%). All the studied traits exhibited high H^2, ranging from 85% to 94% (Table 3.3). Erucic and stearic acids shared the highest H^2 (0.94), followed by palmitic acid (0.91), which significantly exceeded the previously reported H^2 of 50% for *B. napus* (Khan et al. 2008) and 66% for erucic acid in *B. carinata* (Nauman *et al.*, 2021).

These high H^2 estimates observed may be collectively attributed to the predominance of additive genetic variance (Redda *et al.,* 2022), minimal environmental influence on trait expression (Khan et al. 2008), utilization of germplasm with a broad genetic base (Nauman *et al.,* 2021), and the potential impact of strong selection pressures applied in previous breeding efforts (IAEA 1994). These results indicated a strong genetic influence on the studied traits in *B. carinata*, suggesting promising prospects for rapid genetic gain through effective selection breeding in this species. Fekadu (2021) reported similarly high H^2 estimates for most fatty acid- and oil content traits.

GAM provides a quantitative measure of the expected genetic gain for a given trait relative to the population mean (Wright, 1921). It is classified as low (0–10%), moderate (10–20%), or high (>20%) (Johnson et al., 1955). Traits with different GAM levels are expected to yield varying genetic gains (Xu *et al.,* 2020). Breeders utilize GAM to prioritize traits for selection, with higher GAM values indicating greater potential for genetic improvement (Verardi *et al.*, 2011). In this study, oleic, stearic, and eicosenoic acids exhibited moderate GAMs ranging from 14.90–19.87% (Table 3.3), indicating some potential for improvement. Erucic, α-linolenic, palmitic, and linoleic acids, along with oil content, presented high GAMs (22.30–59.29%) at 5% selection intensity, highlighting their exceptional potential for genetic improvement. Varying levels of GAM indicate that the rate of genetic gain may differ among traits.

The integration of GAM with other selection criteria, such as economic value and consumer preference, enhances the relevance of breeding programs (Covarrubias-Pazara, 2020). Furthermore, as H^2 influences the potential for genetic gain and the efficiency of selection strategies, breeders should prioritize traits with high GCV, H^2, and GAM values (Allard, 1960) to optimize genetic improvement and ensure alignment with market demand. Traits with high H² values indicate that a significant portion of the phenotypic variation is due to genetic factors, making them more amenable to selection (Verardi *et al.,* 2011). High H² values often correlate with a higher GAM, enhancing the predictability of genetic gains (Johnson *et al.,* 1955). Traits exhibiting high values across all three metrics have been reported to be the most conducive for improvement (Gudi *et al.,* 2022). In this context, α-linolenic acid has emerged as promising compound because of its high GCV, H², and GAM (Table 3.3). Additionally, linoleic and palmitic acids had high H² and GAM values with moderate GCVs, suggesting that these traits have substantial potential for *B. carinata* improvement through selection or hybridization approaches. Consistent with these results, Fekadu (2021) reported high GCV, H², and GAM estimates for α-linolenic acid, linoleic acid, and palmitic acid.

Table 3.3: Estimates of variance components, heritability, and genetic advance for eight traits in the *B. carinata* germplasm studied

Traits	δ^2g	δ^2ph	GCV	PCV	H^2	GA	GAM
Oil content (%)	4.73	5.55	5.01	5.44	0.85	19.88	45.94
Erucic acid (%)	14.83	15.86	7.86	8.12	0.94	29.01	59.20
Linoleic acid (%)	3.56	4.16	11.66	12.58	0.86	3.62	22.30
α-linolenic acid (%)	8.05	9.06	20.41	21.63	0.89	7.35	52.84
Oleic acid (%)	0.72	0.84	7.80	8.45	0.86	1.62	14.90
Eicosenoic acid (%)	0.71	0.82	10.28	11.14	0.87	1.62	19.87
Palmitic acid (%)	0.32	0.35	16.68	17.26	0.91	1.12	32.63
Stearic acid (%)	0.17	0.20	27.91	28.56	0.94	0.28	18.40

δ^2g = Genotypic variance, δ^2ph = Phenotypic variance, GCV: Genotypic coefficient of variation, PCV: Phenotypic coefficient of variation, H^2: Broad sense heritability, GA: Genetic advance, GAM: Genetic advance as percent of mean.

3.3.5.3. Genotypic Correlations of Traits

Genotypic correlations among eight traits in *B. carinata* were examined to identify candidates for indirect selection, potentially improving economically valuable traits. The analysis revealed significant positive genotypic correlations for most traits (Figure 3.2), suggesting that selecting for one trait could indirectly influence others. Key findings include: strong positive correlation between α-linolenic acid and linoleic acid (r = 0.86), indicating that selection for increased α-linolenic acid content could lead to an indirect increase in linoleic acid levels. Erucic acid had positive correlations with linoleic acid (r = 0.18), eicosenoic acid (r = 0.16), and α-linolenic acid (r = 0.11) while negative correlations with oil content (r = -0.11) and palmitic acid (r = -0.15). These findings suggest that breeding for higher erucic acid contents could have a positive effect on linoleic, eicosenoic, and α-linolenic acids, and a negative effect on oil content and palmitic acid traits. Similarly, eicosenoic acid showed positive correlation with oleic acid (r = 0.15) and negative correlation with α-linolenic acid (r = -0.18). Stearic acid exhibited positive correlations with both linoleic acid (r = 0.19) and α-linolenic acid (r = 0.19).

These results offer valuable insights for breeding programs, enabling the development of strategies for the simultaneous improvement of multiple traits through indirect selection. This approach has the potential to accelerate genetic progress compared with direct selection alone. The importance of genetic associations between traits for understanding the effectiveness of indirect selection in breeding programs has been previously reported (Verardi, 2011). These findings align with those of prior studies on canola (Khan *et al.*,

2008) and *B. carinata* (Fekadu, 2021), which reported positive genotypic correlations between erucic acid, linoleic acid, and α-linolenic acid. Implications of the findings for breeding strategies include simultaneous improvement of α-linolenic and linoleic acids through selection for either trait. Careful consideration of the trade-offs between erucic acid content and oil content/palmitic acid levels. Potential for developing cultivars with specific fatty acid profiles by leveraging the observed correlations. These insights can guide breeders in developing more efficient selection strategies, potentially leading to faster development of *B. carinata* cultivars with improved oil quality traits tailored to specific industrial or nutritional applications.

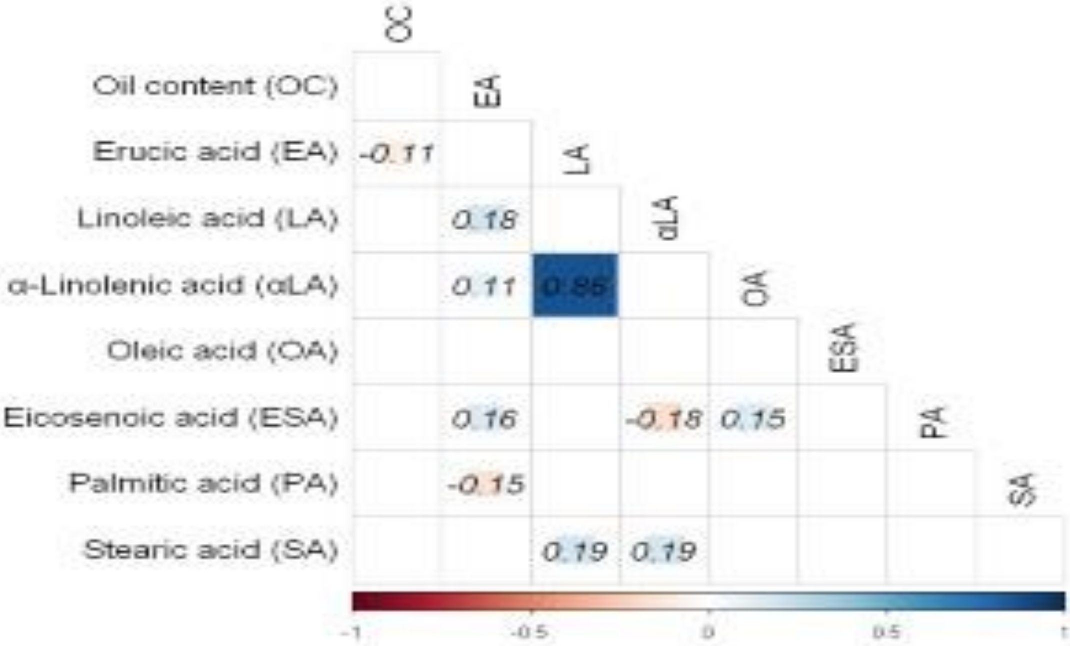

Figure-3.2 Pearson's correlation coefficient for eight traits of *B. carinata* tested at HARC

Squares with numerical values indicate statistically significant relationships at $p \leq 0.05$, empty squares represent non-significant correlations $p > 0.05$

3.3.6. Cluster Analysis

This study assessed the genetic diversity and clustering patterns of 386 *B. carinata* accessions using cluster analysis. The UPGMA algorithm effectively categorized the accessions into seven distinct clusters (Figure 3.3). These results align with previous studies reported on *B. carinata* (Abebe *et al.*, 2009; Genet *et al.*, 2005), with little variations likely attributable to differences in germplasm collections.

The analysis showed cluster 1 (C1), the largest cluster encompassed 359 accessions (93%) from diverse Ethiopian regions including Amhara: 109, Benishangul Gumuz: 12, Central Ethiopia: 4, Harari: 4, Oromia: 197, South Ethiopia: 12, Southwest Ethiopia: 8 and Tigray: 13 (Figure 3.3; Table 3.4). This revealed a limited association between geographic origin and genetic diversity, suggesting that genetic factors exert a stronger influence than geographical location in shaping their diversity patterns. Conversely, clusters 2 to 7 (C2–C7) exhibited smaller and more geographically restricted distributions. These results corroborate prior research on Brassica species (Genet et al. 2005; Khan et al. 2008; Fekadu, 2021) and other plant species (Yi *et al.,* 2011; Hodkinson *et al.,* 2012; Liu *et al.,* 2014), emphasizing the dominant influence of genetic factors over geographic location on diversity patterns. The observed cross-regional clustering in C1 and little in C2, C3, C4, and C7 (Figure 3.3) suggests shared genetic ancestry or potential germplasm exchange among Ethiopian regions. This finding is particularly significant as it indicates a complex history of genetic flow and admixture within the *B. carinata* population in Ethiopia.

Conversely, the intraregional sub-clustering observed within C5 and C6 might reflect local adaptation processes or historical recombination events, pointing to the existence of unique, regionally-specific genetic variants. These clustering patterns are consistent with studies on *B. carinata* (Hunde *et al.,* 2013), soybean (Fu *et al.,* 2015), and foxtail millet (Li *et al.,* 2019), which reported cross-regional clustering due to germplasm movement and shared ancestry. The presence of both cross-regional and intraregional clustering in our study provides a nuanced picture of *B. carinata's* genetic structure in Ethiopia, reflecting a balance between broad genetic exchange and localized adaptation.

The dominance of cluster C1, encompassing 93% of the accessions, warrants further investigation. This large cluster might represent a core gene pool of *B. carinata* in Ethiopia, possibly reflecting the crop's domestication history or widespread cultivation practices. The smaller, more geographically restricted clusters (C2–C7) could represent divergent lineages or locally adapted populations, potentially harboring unique alleles of agronomic importance. From a breeding perspective, these results offer valuable insights for developing targeted strategies. The extensive genetic diversity within C1 suggests that this cluster could serve as a primary gene pool for breeding programs aiming to improve broadly adapted varieties. Conversely, the smaller clusters might be sources of novel traits or adaptations to specific environmental conditions, making them valuable for trait introgression or developing regionally optimized cultivars.

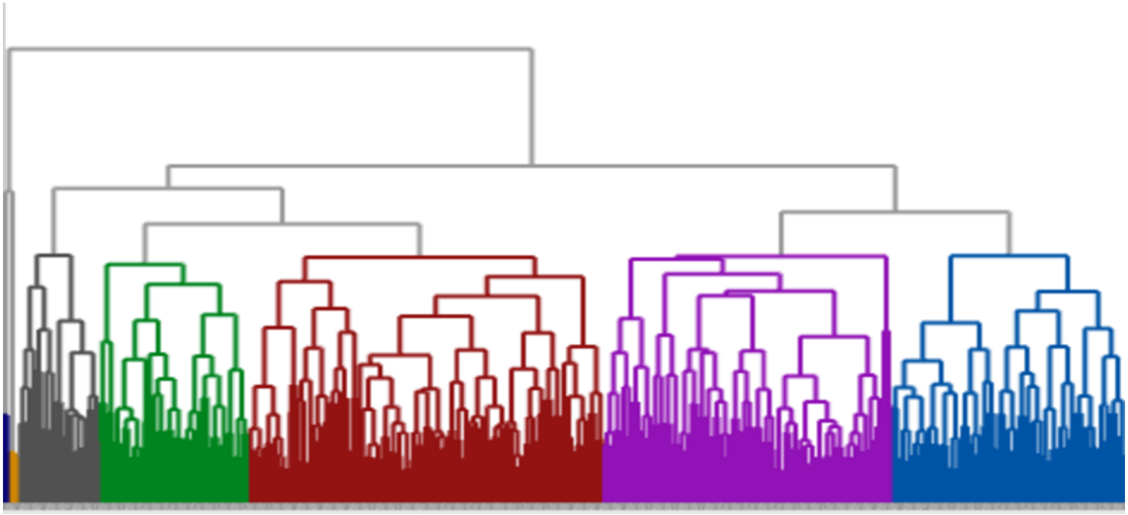

Figure-3.3 Dendrogram of the 386 *B. carinata* accessions constructed on the basis of oil content and fatty acid profile traits

3.3.6.1. Intra- and Inter-Genetic Distance Analysis

Genetic distance analysis among the seven *B. carinata* accession clusters revealed significant divergence, particularly between clusters C1, C2, C3, C4, and C7 (Table 3.4). The pairwise genetic distances exhibited high divergence between C1–C7 (13.95), C2–C7 (13.93), C3–C7 (13.13), and C4–C7 (11.41) whilst C2–C3 (4.55) whilst C3–C4 (5.12) had comparatively lower divergence. These substantial intercluster distances, particularly between C1–C7, C2–C7, C3–C7, and C4–C7, suggest significant potential for heterosis (hybrid vigor) through strategic hybridization of accessions from these divergent clusters. Crosses between accessions from these highly divergent clusters may yield superior hybrids exhibiting heterosis for agronomically important traits. Our findings align with those of Genet et al. (2005), who reported significant intercluster divergence between C1–C7, C2–C7, and C4–C7 in *B. carinata*.

Intra-cluster genetic distances also revealed interesting patterns including high intra-cluster variation within C1 (3.62) and C3 (3.51) and lower intra-cluster variation within the other clusters (Table 3.4). The substantial intra-cluster genetic distances within C1 and C3 suggest considerable genotypic variation compared to other clusters. This diversity implies a potentially wider range of oil content and fatty acid profiles available within these clusters offers opportunities for selecting Top performing accessions with desirable oil content and fatty acid profiles. The notably high mean genetic distance within C7 (5.42) further underscores the overall significant genetic diversity present among *B. carinata* accessions.

The observed high overall patterns of inter- and intra-cluster diversity emphasizes the importance of maintaining and characterizing *B. carinata* germplasm collections to ensure the availability of diverse genetic resources for future breeding efforts. By leveraging this genetic diversity strategically, breeders can potentially develop improved *B. carinata* cultivars with enhanced yield, oil quality, and adaptability to diverse environmental conditions. Although using accessions from different clusters can introduce valuable genetic diversity and heterosis, it also poses challenges related to genetic compatibility, linkage drag, and resource allocation. Breeders need to strike a balance between genetic distance and compatibility to maximize the benefits of crossing diverse accessions in their breeding programs. These results reinforce the established concept that exploiting genetic diversity, both between and within populations, can maximize heterosis in breeding programs (Rahman *et al.*, 2013; Hallauer *et al.*, 2010; Du *et al.*, 2019).

Table 3.4: Intra- (bold diagonal) and inter- (off-diagonal) cluster distances, along with mean distances and cluster sizes, in *B. carinata* clustering analysis on the basis of oil content and fatty acid traits

Cluster	C1	C2	C3	C4	C5	C6	C7	Mea Distance	Number of accessions
C1	**3.62**							3.31	359
C2	9.33	**2.95**						3.13	11
C3	6.69	4.55	**3.51**					2.85	3 (113, 345, 364)
C4	5.57	5.18	5.12	**3.03**				3.22	6
C5	6.17	10.43	8.18	6.06	**2.15**			3.67	2 (55, 95)
C6	9.34	5.67	4.70	5.88	7.36	**1.15**		3.45	3 (15, 38, 84)
C7	13.9	13.93	13.13	11.41	8.16	9.28	**2.08**	5.42	2 (101, 377)

The abbreviations C1, C2, C3, C4, C5, C6, and C7 represents clusters 1, 2, 3, 4, 5, 6 and 7, respectively.

3.3.6.2. Analysis of the Means of Traits among Clusters

Analysis of cluster means revealed significant variation in fatty acid composition and oil content among the seven *B. carinata* clusters (Table 3.5). The clusters exhibited distinct profiles such as C1: highest oil content (45.57%), highest erucic (51.65%) and oleic (11.85%) acids and the lowest linoleic (11.01%), α-linolenic (9.12%), palmitic (1.12%) acids. C2 displayed lowest erucic (36.31%), eicosenoic (4.01%) and stearic (1.07%) acids. Clusters, C3 to C7 exhibited moderate to high variation across all traits (Table 6) with oil content range: 37.04–42.36%; erucic acid range: 43.50–50.19%; linoleic acid range: 12.26–17.92%; α-linolenic acid range: 12.56–19.6%; oleic acid range: 5.05–9.44%, eicosenoic acid range: 4.12–9.7%; palmitic acid range: 2.27–3.38% and stearic acid range: 1.48–2.38% (Table 3.5).

These observed diversity in oil content and fatty acid profiles provides a robust foundation for developing improved *B. carinata* cultivars tailored to various market demands and industrial applications. Specifically, the high oil and specific fatty acid profiles in C1 accessions, characterized by high oil content and elevated erucic and oleic acid levels, are valuable resources for breeding programs aimed at high oil production and specific fatty acid profiles. The low erucic acid varieties in C2 accessions, exhibiting reduced levels of erucic, eicosenoic, and stearic acids, could serve as parental lines for breeding low erucic acid varieties, which are desirable for certain culinary and industrial applications. The diverse fatty acid profiles in clusters 3 to 7, displaying moderate to high variation across all traits, represent valuable genetic resources for the strategic selection of parental lines with desired fatty acid profiles. By strategically leveraging this genetic variation, breeders can potentially: enhance oil yield and quality for biofuel production; develop varieties with optimized fatty acid profiles for specific culinary or industrial uses and improve the nutritional value of *B. carinata*-derived products.

All in all, this comprehensive characterization of fatty acid diversity within *B. carinata* clusters offers valuable insights for designing effective breeding strategies and highlights the importance of maintaining diverse germplasm collections for future crop improvement efforts. These results corroborate previous research by Mnzava et al. (2007), Abebe et al. (2009) and Fekadu (2021), who reported moderate to high mean cluster values for oil content and other fatty acids in *B. carinata*.

Table 3.5: Mean values of seven clusters for eight traits of the 386 *B. carinata* accessions

Traits/Cluster	C1	C2	C3	C4	C5	C6	C7
Oil content (%)	45.57	43.48	40.04	42.36	39.04	41.38	37.04
Erucic acid (%)	51.65	36.31	43.50	47.41	46.54	50.19	48.54
Linoleic acid (%)	11.01	11.32	12.41	17.92	12.26	13.48	15.86
α-linolenic acid (%)	9.12	11.30	17.90	16.89	12.56	14.70	19.60
Oleic acid (%)	11.85	5.26	8.41	6.15	7.39	9.44	5.05
Eicosenoic acid (%)	5.07	4.01	5.15	6.28	4.12	8.76	9.70
Palmitic acid (%)	1.12	1.20	3.38	2.27	2.50	2.47	2.88
Stearic acid (%)	1.33	1.07	1.82	1.61	1.55	1.48	2.38

The abbreviations C1, C2, C3, C4, C5, C6, and C7 represents clusters 1, 2, 3, 4, 5, 6 and 7, respectively.

3.4. Conclusion and prospects

This comprehensive study of 386 *B. carinata* accessions revealed highly significant variation ($p \leq 0.001$) in seed oil content and fatty acid composition, highlighting the rich genetic diversity within the evaluated germplasm. Key findings include: top performed 19 accessions: acc-386, acc-385, acc-384, acc-383, acc-382, acc-381, acc-380, acc-378, acc-379, acc-377, acc-376, acc-375, acc-374, acc-373, acc-372, acc-371, acc-370, acc-369, and acc-368 exhibited superior traits such as oil content (43.66–47%) and erucic acid content (52.4–56.57%) that substantially higher than population and checks means. High GAM (22.30–59.29%) coupled with high H^2 (85% to 94%) estimates for several fatty acids and oil content indicate substantial breeding potential. Strong positive genotypic correlations particularly α-linolenic acid with linoleic acid (r = 0.86) and erucic acid (r = 0.11), linoleic acid with erucic acid (r = 0.18) suggest potential for indirect improvement of linolenic and erucic acids through selection for α-linolenic acid. Cluster analysis identified seven distinct diversity groups with the largest cluster, C1, encompassed 359 accessions (93% of total), representing diverse germplasm collections. High intercluster distances were observed between C1–C7 (13.95), C1–C3 (13.93) and C1–C2 (13.13) indicate potential for exploiting hybrid vigor through crosses between divergent groups.

Based on these findings, the study propose the following strategies for *B. carinata* improvement: accessions acc-386, acc-02, acc-385, and 309 show significant potential to improve overall oil content, erucic acid levels, α-linolenic acid content, eicosenoic acid content, and stearic acid content for industrial applications. Accessions acc-372, acc-326, and acc-270 are promising candidates for developing cultivars with higher linoleic acid content, oleic acid content and palmitic acid content for edible oil and nutritional enhancement.

In general, this study underscores the importance of conserving and utilizing genetic diversity to enhance the quality and versatility of Brassica oilseed crops. The identified Top performing accessions and observed genetic relationships provide a robust foundation for future breeding efforts aimed at developing *B. carinata* cultivars with tailored oil profiles to meet evolving industrial and nutritional demands. In the future, the environmental influences on oil content and fatty acid profiles to develop stable, high-performing cultivars across diverse growing conditions should be studied. Moreover, the application of high-throughput genotyping techniques could gain deeper insights into the genetic mechanisms governing seed oil content and fatty acid composition in *B. carinata*.

CHAPTER 4

4. Uncovering Genetic Diversity and Population Structure in Ethiopian Mustard (*Brassica carinata* A. Braun) Germplasm Using DArTSeq-Generated SNPs

Abstract

Ethiopian mustard (Brassica carinata A. Braun) is a promising oilseed crop with the potential for sustainable biofuel and bio-industrial applications. Despite the presence of diverse germplasms in Ethiopia, their genetic diversity remains largely unexplored. This study evaluated the genetic diversity and population structure of 188 B. carinata accessions using high-density Single Nucleotide Polymorphism (SNP) markers generated though DArTseq™ Genotyping-by-Sequencing (GBS). Of the 15,515 identified DArTSeq SNPs, 3,793 high-quality markers were retained and used to analyze the genetic diversity and population structure. The results from STRUCTURE, principal coordinate analysis (PCoA), and neighbor-joining tree analyses revealed two slightly distinct subpopulations, with Pop1 predominantly comprising accessions from the Oromia and Amhara regions (86.17%), whereas Pop2 primarily consisted of released varieties, suggests the influence of targeted selection. Despite the presence of subpopulations, PCoA indicated a relatively limited overall genetic diversity among the accessions. Analysis of Molecular Variance (AMOVA) revealed higher genetic variation within populations (65.19%) than between populations (34.81%), resulting in low genetic differentiation (PhiPT = 0.02) and high gene flow (Nm = 5.74). Notably, subpopulation formation was not strongly correlated with geographical origin, highlights that factors beyond geography, such as gene flow and selection pressure, may have played a significant role in shaping the observed genetic diversity. Genetic diversity indices revealed a slightly low-to-moderate variation within the B. carinata populations, as evidenced by the slightly low expected heterozygosity (He = 0.21) and moderate polymorphic information content (PIC = 0.36). Overall, this study revealed a moderate level of genetic diversity within the evaluated B. carinata accessions. The results offer valuable insights into the genetic structure of this species and highlight the need for targeted strategies to enhance genetic diversity in future breeding initiatives and conservation efforts.

***Keywords**: Brassica carinata*; Germplasm; Genetic Diversity, Population Structure; SNPs Marker

4.1. Introduction

Ethiopian mustard (*Brassica carinata* A. Braun) is a versatile oilseed crop native to Ethiopian highlands and surrounding regions with a 4000-year cultivation history (Getinet *et al.,* 1996). This amphidiploid species (genome BBCC, n=17) evolved through the natural hybridization of *B. nigra* (BB, n=8) and *B. oleracea* (CC, n=9), followed by chromosome doubling (Prakash and Hinata, 1980). Over three million Ethiopian smallholder farmers cultivate *B. carinata* primarily for its leaves and oil-rich seeds (Alemayehu and Becker, 2002). Beyond its traditional uses, the crop's adaptability to diverse climates, its potential contribution to global food security (Seepaul *et al.,* 2021), and its applications in the biofuel and oleo-chemical industries have spurred its worldwide introduction (Tesfaye *et al.,* 2023). The promising properties of *B. carinata* extend to sustainable aviation fuel production owing to its high erucic acid content (31–46%), low saturated fatty acids, and ease of refining, potentially reducing greenhouse gas emissions (Seepaul *et al.,* 2019; George *et al.,* 2021). Furthermore, the crop is resilient to environmental stressors, disease resistance, high seed yield potential on marginal lands, and a large seed size (Bulcha *et al.,* 2017). This multifaceted crop has gained recognition across various sectors, including agriculture, aviation, pharmaceuticals, plastics, and bioenergy (Rahel *et al.,* 2020, Yirssaw *et al.,* 2024).

Ethiopia, recognized as the center of origin and diversity for *B. carinata* (http://www.ebi.gov.et), boasts a rich germplasm collection maintained within the Ethiopian Biodiversity Institute (EBI) and various agricultural research institutions (http://www.eiar.gov.et). This diverse genetic resource provides a valuable foundation for the development of improved varieties (Takele, 2022). Over the past four decades, significant research has focused on enhancing the yield, quality, and secondary metabolite traits (Misteru *et al.,* 2013; Kumar *et al.,* 2021; Li *et al.,* 2022). However, despite these breeding efforts, the full genetic potential of *B. carinata* remains largely unknown, owing to a limited understanding of its genetic diversity. Genetic diversity is pivotal for plant adaptation and evolution, and drives the development of improved crop varieties with desirable traits (Azeez *et al.,* 2018). Germplasm characterization plays a crucial role in identifying variations in breeding and guiding strategies for collection, conservation, and sustainable use (Li *et al.,* 2022). Previous studies using agro-morphological, physiological, and biochemical markers have revealed considerable genetic variation (Yared *et al.,* 2010; Tesfaye *et al.,* 2014; Fekadu, 2021). However, these markers often lack precision owing to their environmental and developmental influences. Molecular marker-based analyses offer a more robust approach for detailed genetic characterization (Li *et al.,* 2022). Earlier genetic diversity studies in *B. carinata* employed a range of markers, from early stage techniques, such as Random Amplification Polymorphic DNA (RAPD) (Peng *et al.,* 2023) and Amplified Fragment Length Polymorphism (AFLP) (El-Esawi *et al.,* 2016), to more advanced markers, such as Simple Sequence Repeat (SSR) (Thakur et al., 2021) and Single Nucleotide Polymorphism (SNP) (Tesfaye *et al.,* 2023). Despite their advantages, these markers often suffer from limitations such as low density and incomplete coverage (Thakur *et al.,* 2019; Khedikar *et al.,* 2020), hindering comprehensive genetic diversity analyses. To address these limitations, the Diversity Array Technology (DArT) emerged in the early 2000s (Valdisser *et al.,* 2017).

High-throughput DArTSeq SNP marker technology has significantly advanced research on plant genetic diversity and population structure (Mijangos *et al.,* 2022). These markers are characterized by genome-wide abundance, ease of replication, reliability, comprehensive genome coverage, and suitability for large-scale genotyping (Fiust *et al.,* 2015). Although successfully applied to various crops, including sorghum (Muhammed *et al.,* 2023; Phoebe *et al.,* 2023), barley (Matties *et al.,* 2012), wheat (Laido *et al.,* 2013), macadamia (Alam *et al.,* 2018), and maize (Adu *et al.,* 2019), DArTSeq SNP marker technology remains unexploited in *B. carinata* diversity studies. The lack of such robust genome-wide data has restricted previous *B. carinata* studies to parent selection based on phenotypic characteristics or limited old molecular marker data, which has led to limited estimation of the true extent of genetic diversity and inaccurate population structure inference within and among *B. carinata* populations. This constraint has significantly hindered the implementation of large-scale genotyping initiatives, which are essential for the comprehensive assessment of genetic diversity and population structure across a broad spectrum of accessions. As a result, the development of improved cultivars and the full realization of the agronomic and economic potential of *B. carinata* have been impeded. Therefore, addressing this critical research gap is fundamental for the advancement of breeding programs and the sustainable exploitation of this valuable crop. Hence, this study aimed to use high-density DArTSeq-generated SNP markers to evaluate the genetic diversity of Ethiopian mustard germplasm. Specifically, this study aimed to (i) assess genetic diversity within the germplasm and (ii) analyze population structure and genetic relationships among accessions. These findings will contribute to breeding programs for developing improved varieties, highlighting the importance of genetic diversity for improving and supporting the conservation of this valuable genetic resource.

4.2. Materials and Methods

4.2.1. Genetic Materials

This study assessed the genetic diversity of 188 *B. carinata* germplasms collected from various Ethiopian regions between 1984 and 2022 (http://www.ebi.gov.et; http://www.eiar.gov.et/holetta/). The majority of the accessions (160) were sourced from EBI (http://www.ebi.gov.et). The remaining 28 accessions, including 23 accessions and five released varieties (Derash, Holetta-1, S-67, Tesfa, and Yellow Dodola), were supplied by the Holeta Agricultural Research Center (HARC). Relevant passport data, some morphological, seedoil content and fatty acid attribute data for all germplasm collections are given in Appendix Table 2.

4.2.2. Genomic DNA Extraction and DArT Genotyping

Five seeds per accession were sown in seedling trays at the School of Plant and Horticultural Sciences Research Facility of Hawassa University, Ethiopia. Three-week-old leaf samples were collected from five seedlings of each accession, pooled, and desiccated using silica gel. Ten milligrams of dried leaf tissue from each accession were subsequently shipped to the SeqArt Africa laboratory at the International Livestock Research Institute (ILRI), Kenya, for genotyping analysis. Genomic DNA (gDNA) was isolated from the dried leaf samples using a Nucleomag Plant Kit (Macherey-Nagel, Germany). The quality and quantity of the gDNA were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). To further verify gDNA integrity, the samples were electrophoretically separated on a 0.8% agarose gel stained with ethidium bromide in 1x TBE buffer at 70 V for 45 min.

Forty microliters of gDNA (50 ng/µL) per accession were used for whole-genome scanning with DArTseq™ Genotyping by Sequencing (GBS) technology (https://www.diversityarrays.com/). The GBS protocol involves library construction encompassing restriction enzyme digestion, adaptor ligation, and PCR amplification to generate sequence-ready libraries. These libraries were subjected to single-end sequencing on an Illumina HiSeq2500 platform, which generated 69 base reads per sample. Sequencing reads were aligned to the reference genome of *B. carinata,* GCA_016771965.1_ASM1677196v1 https://www.ncbi.nlm.nih.gov/ datasets/ genome/GCA_016771965.1) (Song *et al.,* 2021) using the DArTsoft14 algorithm to identify the SNPs. As co-dominant markers, the SNPs were encoded in a binary matrix. The presence of a specific SNP allele was coded as "1," while its absence was coded as "0." Homozygous and heterozygous accessions were represented as "1/1" or "0/0" and "1/0," respectively.

4.2.3. Statistical Analysis

(i) SNP Filtration and Genetic Properties: SNP quality control was performed using the dartR package in R (Gruber *et al.,* 2018). This process removed loci with low call rates (<70% for markers and <50% for individuals), low reproducibility (<95%), monomorphism (no variation), missing data (entire loci with NA scores or SNPs exceeding 20% of missing accessions), and low minor allele frequency (MAF < 0.05). Only high-quality SNPs were used for further analyses (Matties *et al.,* 2012). The genetic properties of the filtered SNPs were assessed using three parameters: observed heterozygosity (Ho), the proportion of individuals heterozygous for a particular SNP; Expected heterozygosity (He), the expected proportion of heterozygotes assuming Hardy-Weinberg equilibrium; and polymorphic information content (PIC), a measure of the informativeness of an SNP for diversity analysis (Alam *et al.,* 2018).

(ii) Genetic Diversity Analysis: Genetic diversity within populations was estimated using several indices calculated using the gl.report.heterozygosity function in dartR (Mijangos *et al.,* 2022). These included minor allele frequency (MAF)-frequency of the less common allele at a given SNP, Major allele frequency (MaF)-the frequency of the more common allele at a given SNP. Ho, He, and PIC.

(iii) Population Structure and Genetic Relationship Analysis: Population structure was analyzed using Bayesian clustering implemented in STRUCTURE v2.3.4 (Earl and vonHoldt, 2012). This software assigns individuals to genetic groups (K) ranging from 2 to 10. Each K value was run five times with extensive burn-in and 100,000 Markov Chain Monte Carlo (MCMC) iterations to ensure convergence. The optimal K value was determined using the Evanno method (Evanno *et al.,* 2005). Clustering results were visualized using DISTRUCT v1.1 (Rosenberg, 2004) after post-processing with CLUMPP v1.1.2 (Jakobsson and Rosenberg, 2007). Individuals with high membership coefficients (≥ 0.70) were assigned to distinct populations, whereas the others were considered admixed.

(iv) Phylogenetic relationships: To further explore genetic relationships, pairwise fixation indices were calculated using the StAMPP package (Pembleton *et al.,* 2013). Additionally, neighbor-joining trees were constructed based on a Euclidean distance (Kamvar *et al.,* 2015) dissimilarity matrix using the dartR package Core Team, 2023). A dendrogram was generated using the unweighted pair group method with arithmetic mean (UPGMA) clustering algorithm employing the ggdendro and ggplot2 packages (Letunic and Bork, 2021) in R.

(v) Principal Coordinate Analysis (PCoA) was performed using the Eigenstrat method (Price *et al.*, 2006) and visualized using ggplot2.

(vi) Population Differentiation and Gene Flow: Analysis of Molecular Variance (AMOVA) was conducted using the poppr package (Kamvar *et al.*, 2014) to quantify genetic differentiation among populations and estimate gene flow. This involved calculating Nei's genetic distance (Nei, 1972) between the populations. The fixation index (FST) and haploid number of migrants (Nm) were obtained from AMOVA analysis which provide insights into the degree of population differentiation (FST) and the level of gene flow (Nm) between populations.

4.3. Results and Discussion

4.3.1. Analysis of Markers Distribution and Density

Comprehensive genotyping analysis of 188 *B. carinata* accessions was conducted using a high-density DArTSeq SNP genotyping platform, resulted in 15,515 markers. Of these, 3,793 SNPs exhibited polymorphisms across the entire genome (Appendix Table 3). These polymorphic SNPs were distributed across all 17 chromosomes, 8 from the B genome and 9 from the C genome (Figure 4.1a). Visualization of SNP density within a 1 MB window revealed a notable concentration, particularly on chromosome B1, highlighted in green in the top bottom of Figure 4.1b. This chromosome exhibited the highest number of SNP markers (376), whereas chromosome C9 displayed the lowest number (103) (Figure 4.1 b). Overall, the B genome exhibited a slightly higher proportion of SNPs (56.85%) than the C genome (43.15%) (Appendix Table 4.2), which is consistent with the results of previous studies by Zou et al. (2014) and Khedikar et al. (2020). This disparity likely reflects the earlier divergence of the B genome (8 million years ago compared to 4 million years ago for the C genome), which might allow for greater mutation accumulation and potentially functional differences, such as higher gene density related to adaptation (Lysak et al., 2005).

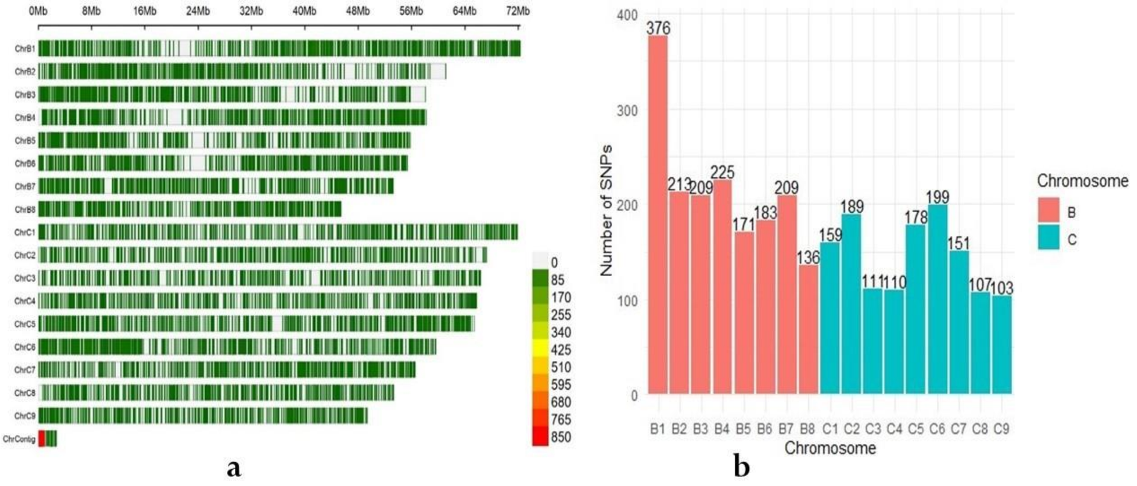

Figure-4.1 Density (a) and Genome-wide distribution (b) of 3,793 DArTSeq SNPs across 17 B. carinata chromosomes within a 1 megabases (Mb) window size. The different colors (a) represents SNP density and marker quantity within a 1 Mb window size.

Analysis of single nucleotide polymorphisms (SNPs) revealed a distinct pattern in the distribution of transitions and transversions (mutational bias) in *B. carinata* B and C genomes, with a higher frequency of transitions than transversions, particularly within the B genome (Table 4.1). Transitions (C/T, T/C, A/G, G/A) represented 37.83% of the B genome SNPs, which was significantly higher than the 24.49% in the C genome SNPs. Conversely, transversions accounted for 24.23% of SNPs in the B genome. Furthermore, all four possible transversion SNPs (A/C, C/A, G/T, and T/G) were exclusively observed within the B genome. In contrast, both genomes exhibited only two types of transition SNPs. These findings suggest that transition mutations are more prevalent than transversions in the studied genomes, particularly in the B genome, may result from mutational bias, genetic drift, selection pressures, or unique B genome characteristics, such as DNA repair variants or methylation patterns (Wu et al., 2022; Yim et al., 2022). These results are consistent with previous Brassica studies (Park et al., 2009; Wong, 2013; Tesfaye et al., 2023).

Table 4.1: Percentage of transition and transversion DArT SNPs in *B. carinata* genomes

Genome	SNP Type	Base substitutions	No SNP loci	Frequency of SNP (%)	Total No of SNPs	Proportion of SNP (%)
B	Transitions	C/T	746	19.67	1435	37.83
		T/C	689	18.17		
	Transversions	A/C	127	3.35	919	24.23
		A/T	103	2.72		
		C/A	138	3.64		
		C/G	95	2.51		
		G/C	88	2.32		
		G/T	114	3.01		
		T/A	136	3.59		
		T/G	118	3.11		
C	Transition	A/G	429	11.31	929	24.49
		G/A	500	13.18		

4.3.2. Analysis of Genetic Parameters

The analysis of 3,793 DArTseq SNP markers revealed moderate genetic diversity within the *B. carinata* germplasm. (Table 4.2). Expected heterozygosity (He) ranged from 0.15 to 0.31, with an average of 0.24, while observed heterozygosity (Ho) ranged from 0.08 to 0.12, with an average of 0.09. This observed heterozygosity deficit (Ho < He) indicates a moderate level of inbreeding within the population, further supported by an inbreeding coefficient (FIS) of 0.51. This suggests moderate inbreeding despite the species' long cultivation history (Alemayehu & Becker, 2002). Factors contributing to this lower-than-expected diversity may include geographical isolation limiting gene flow (Dixon, 2007) and the species' predominantly self-pollinating nature (Sleper & Poehlman, 2006). The observed heterozygote deficit is consistent with deviations from the Hardy-Weinberg equilibrium, potentially due to non-random mating or selection pressures. Similar findings of low-to-moderate diversity have been reported in *B. carinata* (Khedikar et al., 2020; Tesfaye et al., 2023) and *B. napus* (Rahman et al., 2022).

Polymorphism Information Content (PIC) values ranged from 0.15 to 0.36, with approximately 70.6% of the SNPs exhibited PIC values ≤ 0.36, suggests a moderate level of informativeness (Appendix Table 3). This is consistent with previous studies on *B. carinata* (Tesfaye et al., 2023), but lower than that reported for *B. rapa* (Ramchiary et al., 2011). According to Botstein et al. (2007), PIC values above 0.5 are considered highly informative, those between 0.25 and 0.5 are moderately informative, and those below 0.25 are slightly informative. Hence, these PIC values suggest moderate polymorphism.

The minor allele frequency (MAF) ranged from 0.08 to 0.12, with an average of 0.10, while the major allele frequency (MaF) ranged from 0.09 to 0.15, with an average of 0.12, reflects a relatively balanced distribution of alleles, and the major allele was generally more common than the minor allele. These results highlight the need for strategies to enhance *B. carinata* genetic diversity, including broader germplasm exploration and targeted breeding programs. The observed levels of diversity present opportunities for breeding and conservation but also emphasize the potential for inbreeding depression and the importance of maintaining and increasing genetic variation.

Table 4.2: Summary statistics of diversity indices for 188 *B. carinata* accessions based on 3793 DArTseq SNPs

Statistics	Genetic Parameters				
	He	Ho	PIC	MAF	MaF
Mean	0.236	0.091	0.258	0.098	0.116
Lower	0.154	0.067	0.152	0.078	0.091
Upper	0.311	0.118	0.363	0.116	0.151

He = expected heterozygosity, Ho = Observed heterozygosity, PIC = Polymorphic information content, MAF = Minor allele frequency, MaF = Major allele frequency

4.3.3. Analysis of Population Structure and Genetic Relationship

Population structure analysis, conducted using STRUCTURE, identified two slightly distinct subpopulations within 188 *B. carinata* accessions (Figure 4.2A, 4.2B). Evanno's test, based on the ΔK plot (Figure 4.2A), indicated the presence of two optimal clusters (K = 2), confirms the presence of two subpopulations. Accessions were subsequently assigned to either Population 1 (Pop 1 = green) or Population 2 (Pop 2 = red) (Figure 4.2B and Appendix Figure 1). Pop 1, comprised 83.51% of the accessions (n = 157), primarily sourced from eight Ethiopian regions (Table 4.3), with the highest representation from Oromia (n = 85; 54.14%) and Amhara (n = 48; 30.57%). Pop 2, represented 16.49% of the accessions (n = 31), including all five released varieties and accessions from six regions, excluding Central Ethiopia and Harari. This subpopulation had a diverse regional origin, with Oromia contributed the highest proportion (41.95%), followed by Amhara (29.03%), Benishangul Gumuz (12.90%), Tigray (9.68%), and South and Southwest Ethiopia (each 3.23 %) (Table 4.3). This suggests that factors beyond geographical adaptation, such as historical germplasm exchange or shared ancestry, influenced population structure

(Khedikar et al., 2020). The presence of accessions from the same region in different subpopulations indicated genetic diversity arising from ancestral differences and recombination. Notably, all released varieties were clustered within Pop 2, highlighting the impact of selective breeding. This homogeneity may stem from targeted selection or the widespread dissemination of superior varieties, potentially becoming integrated as landraces. Furthermore, the potential origin of the varieties released from Pop 2 landraces underscores their value as genetic resources. Population structure analysis aids in controlling false-positive marker-trait associations (Eltaher et al., 2018). These findings emphasize the need for strategies to enhance genetic diversity within the *B. carinata* germplasm. This will bolster crop resilience and adaptability to environmental challenges, and ensure sustainable production.

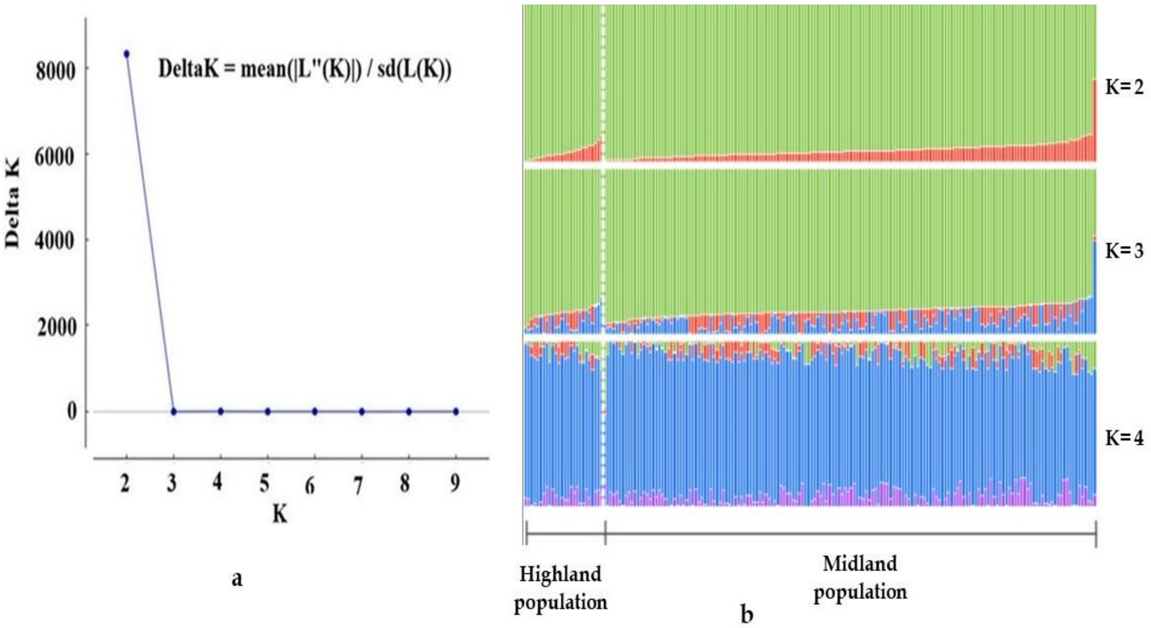

Figure-4.2 Population structure analysis of 188 *B. carinata* accessions. (a) Estimated number of populations based on Evanno’s test (ΔK at K = 2). (b) Bar plot from STRUCTURE analysis for K = 2, K = 3 and K = 4 showing distinct populations.

Table 4.3: Population distribution of the 188 *B. carinata* accessions (Pop1 and Pop2) based on STRUCTURE analysis.

Accession Source	No. Germplasm	Population-1		Population-2	
		Number	%	Number	%
Amhara	57	48	84.21	9	15.79
Benishangul Gumuz	10	6	60.0	4	40.0
Central Ethiopia	3	3	100	0	0
Harari	3	3	100	0	0
Oromia	98	85	86.74	13	13.26
South Ethiopian	5	4	80.0	1	20.0
South West Ethiopia	3	2	66.67	1	33.33
Tigray	9	6	66.67	3	33.33
Total	188	157	86.17	31	16.49

Principal coordinate analysis (PCoA) showed slight genetic differences between 188 *B. carinata* populations, grouping them into two somewhat distinct groups, highland population and midland population (Figure 4.3). This grouping was also seen in the STRUCTURE analysis (Figure 4.2), confirming small genetic differences. These differences accounted for only 7.4% (PCoA 1 = 4.4% and PCoA 2 = 3%) of the total genetic variation, as visualized in the PCoA plot (Figure 4.4, Appendix Table 4).

Additionally, a phylogenetic tree constructed based on genetic similarities showed two slightly different groups, Pop1 and Pop2 (Figure 4.4), corroborating the PCoA and STRUCTURE results.

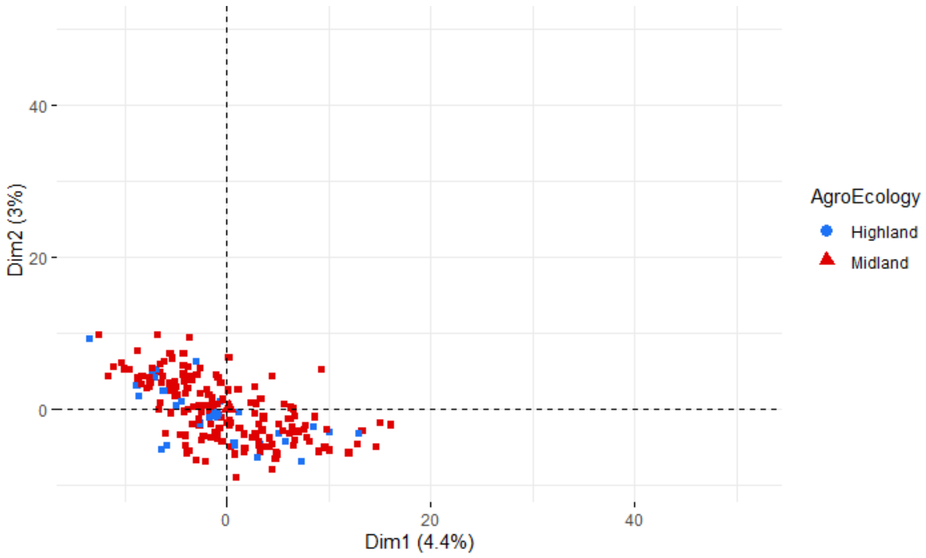

Figure-4.3 The PCoA plot showing clustering of 188 *B. carinata* accessions among two subpopulations, (highland pop = blue, midland pop = red). PC1 and PC2 represent the first and second principal coordinates, respectively, with numbers in parentheses indicating the proportion of the variance explained.

Figure 4.4 Phylogenic tree based on UPGMA showing the two sub-populations clustering, population-1 (yellow) and population-2 (blue) of the 188 *B. carinata* accessions

4.3.4. Analysis of Molecular Variance

Analysis of Molecular Variance (AMOVA) revealed that a significant proportion of the genetic variation (65.19%) resided within populations, whereas only 34.81% was attributable to differences between the two identified subpopulations (Table 4.4). This pattern, with higher within-population than between-population variation, is common in self-pollinating species (Sleper et al., 2006). These results indicate that genetic diversity within *B. carinata* is influenced by factors other than geographic location. This low inter-population differentiation was supported by a low PhiPT value of 0.02 ($p \leq 0.001$) and a high gene flow (Nm = 5.74) (Table 4.4). This Nm values, classified as high gene flow (Nm ≥ 1.0) according to Wright (1969), suggest substantial gene exchange, likely due to pollen and seed dispersal (Eltaher et al., 2018). The observed genetic interconnectedness has practical implications for breeding programs, allowing efficient exploitation of existing genetic variations. Similar findings of low inter-population differentiation have been reported in *B. carinata* (Khedikar et al., 2020; Tesfaye et al., 2023) and *B. napus* (Rahman et al., 2022). Such genetic insights facilitate improved parent selection, marker-assisted selection (MAS), genome-wide association studies (GWAS), and genomic selection (GS). These findings also aid in germplasm identification, conservation, and core collection development, ultimately driving the creation of improved cultivars with enhanced agronomic traits (Bulcha et al., 2017; Lazaridi et al., 2024).

In general, the genetic insights gained from this study have significant practical implications, including facilitating improved parent selection for maximizing hybrid vigor, enhancing the efficiency of marker-assisted selection (MAS), and genome-wide association studies (GWAS) through increased statistical power and optimization of genomic selection (GS) strategies. These findings also enable the identification of valuable new germplasms, refinement of conservation strategies, monitoring of genetic erosion, and the development of representative core collections. Ultimately, this knowledge will drive the creation of novel cultivars with superior agronomic traits such as increased oil yield, enhanced disease resistance, and improved stress tolerance (Lazaridi et al., 2024). Furthermore, this information can empower breeders to refine existing methodologies and optimize the use of existing genetic resources (Bulcha et al., 2017; Lazaridi et al., 2024).

Table 4.4: Analysis of molecular variance (AMOVA), genetic differentiation (PhiPT), and gene flow (Nm) between and within populations of 188 *B. carinata* accessions.

Source	DF	SS	MS	EV	%V	PhiPT	Nm
Between populations	1	0.0015	0.0015	0.00051	34.81	0.02*	5.74
Within populations	187	0.2105	0.0011	0.00071	65.19		
Total	188	0.2120	0.0026	0.00122	100		

* Significant at $p < 0.05$; DF, degree of freedom; SS, sum of square; MS, mean of squares; EV, error of variation; %V, percentage of variation.

4.4. Conclusion

The present study reports a comprehensive genetic diversity analysis of 188 *B carinata* accessions from Ethiopia using high-density DArTSeq SNP markers. Key findings include:

- Of the 15,515 identified DArTSeq SNPs covering 17 chromosomes, 3,793 high-quality markers were retained, provides valuable resources for future diversity studies.
- The population structure analysis revealed two distinct subpopulations (Pop1 and Pop2).
- AMOVA indicated significant genetic variation within populations ($p \leq 0.001$), with relatively low differentiation between subpopulations, supported by a low PhiPT value (0.02) and a high gene flow estimate (Nm = 5.74).
- Notably, subpopulation formation did not exhibit a strong correlation with geographical origin, suggests that other factors such as gene flow, mutation rates, or selective pressures may have played a more significant role in shaping the observed genetic diversity.

Overall, this study revealed a moderate level of genetic diversity within the evaluated *B. carinata* germplasm. The findings provide crucial insights into the genetic structure of this species, laying the foundation for future breeding and conservation efforts. The candidate core collections identified could serve as valuable resources for genome-wide association studies (GWAS) to identify genomic regions associated with important agronomic traits. Furthermore, integrating data from other *B. carinata* collections worldwide would significantly expand our understanding of the entire gene pool, facilitate the identification and conservation of diverse allelic variants of key agronomic traits, ultimately contributing to the development of improved cultivars of *B. carinata*.

CHAPTER 5

5. Summary and Conclusion

Ethiopian mustard (*Brassica carinata* A. Braun), locally known as ‘Gomen zer’. ‘Yehabesha Gomen’ that belongs to the *Brassicaceae* family. It is a promising sustainable source of vegetable oil with potential applications in food production, animal feed, bio-fertilizers, biofuels, cosmetics, oleo-chemicals and the pharmaceutical industry. In addition, this crop is resilient to environmental stressors, disease resistance, and high seed yield potential on marginal lands, which have gained global interest. In Ethiopia, over three million smallholder farmers cultivate it under various agro-ecological zones for food, culinary ingredients, lamp oil, treatment of stomach ailments, and lubricate ‘Enjera’ making pans for over four millennia. Ethiopia, recognized as the center of origin and diversity for *B. carinata*, boasts a rich germplasm collection maintained within the EBI and agricultural research institutions. This diverse genetic resource provides a valuable foundation for the development of improved varieties of *B. carinata*.

Despite its widespread adaptation, availability of abundant germplasm, and significant economic potential as a versatile oilseed crop, the full genetic potential of *B. carinata* remains largely unknown owing to a limited understanding of its genetic diversity. Genetic diversity is pivotal for plant adaptation and evolution and drives the development of improved crop varieties with desirable traits. Hence, the systematic characterization of *B. carinata*, available germplasm, and their attributes is of paramount importance for future tailored breeding and improvement activities. Therefore, this study was designed to assess the morpho-physiological, seed oil composition, and molecular diversity of the *B. carinata* germplasm in Ethiopia.

Three sets of experiments were conducted to achieve these objectives. In the first experiment, 386 *B. carinata* germplasm collections were characterized for quantitative agro-morphological and physiological traits at the Holeta Agricultural Research Center during the 2022 and 2023 summer and autumn (June–December) main cropping seasons using an augmented block design (ABD) with two replicates. The second experiment was carried out to assess the seed oil content and six fatty acid profiles of the 386 *B.carinata* germplasm collections using Nuclear Magnetic Resonance Spectroscopy (NMRS) and Near Infrared Reflectance Spectroscopy (NIRS) respectively at high- and mid-land oilseeds Laboratory, HARC. In the third experiment, the genome-wide molecular characterization of188 *B.carinata* germplasm collections was performed using high-density DArTSeq-generated 3793 SNPs markers at Seqart Africa genotyping laboratory, Nairobi, Kenya.

The Ethiopian mustard accessions exhibited significant phenotypic variation across 27 agro-morphological and physiological traits. This diversity was evident in the wide range of trait means, high heritability, genetic gain as a percentage of mean, principal component analysis (PCA), and cluster analysis results. Notably, 19 Top performing accessions demonstrated substantial increases in seed yield (16.70% to 44.30%) and oil content (27.4 % to 58.7%) compared with both the population mean and checks. PCA revealed that the top five principal components explained over 61% of the variation in leaf size, branching, seed yield, and oil yield contributed most significantly. Cluster analysis identified four groups with substantial genetic distances (8.84–18.21) between accessions, with the highest divergence between C3 and C4. C2 exhibited the highest intra-cluster distance (6.71) and mean genetic distance (5.23), suggests valuable morpho-physiological variation for breeding programs. From this study, two distinct groups of promising accessions were proposed for *B. carinata* improvement based on their unique traits and potential applications. Group-1 (acc-192, acc-386, acc-1, acc-235, acc-294, acc-302, acc-112, acc-331, acc-152, acc-55, and acc-72) demonstrated significant potential to enhance seed yield, oil yield, and quality traits. Group-2 (acc-192, acc-124, acc-377, acc-112, acc-1, acc-386, acc-55, acc-331, acc-235, acc-302, acc-375, acc-72, acc-171, acc-98, and acc-20) exhibited a distinct profile, demonstrating particular suitability for water management strategies.

A comprehensive study of 386 *B. carinata* accessions revealed highly significant variations ($p \leq 0.001$) in seed oil content and fatty acid composition, highlighting the rich genetic diversity within the evaluated germplasm. Key findings include: top performed 19 accessions: acc-386, acc-385, acc-384, acc-383, acc-382, acc-381, acc-380, acc-378, acc-379, acc-377, acc-376, acc-375, acc-374, acc-373, acc-372, acc-371, acc-370, acc-369, and acc-368 exhibited superior traits such as oil content (43.66–47%) and erucic acid content (52.4–56.57%) that substantially higher than population and checks means. High GAM (22.30–59.29%) coupled with high H2 (85% to 94%) estimates for several fatty acids and oil content indicate substantial breeding potential. Strong positive genotypic correlations, particularly between α-linolenic acid and linoleic acid ($r = 0.86$), erucic acid ($r = 0.11$), and linoleic acid and erucic acid ($r = 0.18$), suggest the potential for indirect improvement of linolenic and erucic acids through selection for α-linolenic acid. Cluster analysis identified seven distinct diversity groups, with the largest cluster, C1, encompassing 359 accessions (93% of total), representing diverse germplasm collections. High intercluster distances were observed between C1 and C7 (13.95), C1–C3 (13.93), and C1–C2 (13.13), indicates the potential to exploit hybrid vigor through crosses between divergent groups.

The genetic diversity of 188 *B. carinata* accessions collected from different parts of Ethiopia was assessed using DArTseq SNPs markers. Out of 15515 markers, 3793 informative SNPs were retained and used for genetic diversity and population structure assessment. These polymorphic SNPs were distributed across all 17 chromosomes, including eight from the B genome and nine from the C genome; the B genome contained 56.85% SNPs, whereas the C genome contained 43.15% SNPs. The markers further revealed a distinct pattern in the distribution of transitions and transversions across *B. carinata* B and C genomes, with transition mutations being more prevalent than transversions in the studied genomes, particularly in the B genome.

Different diversity indices, such as expected and observed heterozygosity (He, Ho) and minor and major allele frequencies (MAF, MaF) varied among markers and populations. He ranged from 0.15 to 0.31, with an average of 0.24, while Ho ranged from 0.08 to 0.12, and a mean of 0.09. Ho was consistently lower than He, indicate a moderate level of inbreeding, further supported by an inbreeding coefficient (FIS) of 0.51, implies heterozygote deficiency may be due to restricted cross-fertilization. Approximately 70.6% of the SNPs had a polymorphic information content (PIC) value ≤ 0.13, suggests that a substantial portion of the markers were slightly informative. MAF and MaF had an average of 0.10 and 0.12 respectively, suggests that the major allele was generally more common than the minor allele. Overall, the recorded values for the different diversity indices illustrated slightly lower polymorphism of the markers and the presence of narrow genetic base among the studied accessions.

STRUCTURE analysis based on the Bayesian algorithm identified two sub-populations and showed admixture of populations without clear geographic origin-based grouping. Cluster analysis based on UPGMA using the Euclidian distance matrix grouped the 188 *B carinata* accessions into two major clusters, regardless of their geographic origin. Principal coordinate analysis (PCoA) revealed a clear genetic division within the 188

B. carinata populations, clustering them into two distinct sub-populations, further corroborates the clustering results. In both analyses, accessions collected from different regions were often grouped together and vice versa. The pattern of grouping observed in the PCoA analysis was also aligned with the STRUCTURE and phylogenetic analyses. Analysis of molecular variance (AMOVA) indicated that variations within populations contributed more to genetic variability than those between populations. From the total genetic variation, 65.19% and 44.81% were attributed to variability among individuals within and between populations, respectively.

In general, the morpho-physiological, seed oil content, and fatty acid profiles assessment clearly showed high trait variability, whereas molecular diversity analyses revealed limited genetic bases among the Ethiopian mustard accessions studied. The results also indicated that more variability existed among individual accessions within growing regions than between regions, and accessions clustered sporadically, regardless of their area of growth. This indicates a lack of definite correlation between geographical distance and the pattern of genetic diversity. Lower genetic differentiation among accessions from different growing areas might be due to the presence of genetic material sharing owing to planting material exchange among growers. The exchange of planting materials among growers could also lead to an increase in within-region diversity due to the introduction of new accessions. Hence, the selection of landraces in conservation and breeding work should be based on actual genetic differences, not on the geographic distance of the growing areas. Due to the presence of high-performing accessions for leaf, seed yield, oil yield, and fatty acid profiles over the released varieties, more focus should be given to landrace collections for future conservation, improvement, and commercialization of *B. carinata*.

In conclusion, these findings provide comprehensive information on the extent of genetic variability in *B. carinata* germplasms and provide a foundation for designing efficient collection, conservation, future breeding, and cultivation strategies. Thus, to exploit existing diversity and harmonize improvement activities, the following studies are suggested.

- To develop stable, superior cultivars across diverse growing conditions, the environmental influences on seed yield, oil content, and fatty acid profiles should be studied.
- To identify genomic regions associated with important agronomic and fatty acid profile traits, Genome-Wide Association Studies (GWAS) should be conducted.
- To expand our understanding of the entire gene pool, screen for more variability, identify desirable genes, and establish core collections with diverse allelic variants for conservation and breeding programs, studies should be continued integrating data from other *B.carinata* collections worldwide.

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7. APPENDICES

Appendix Table 2.1: Passport data of *B. carinata* accessions used in the agro-morphological and seed oil composition diversity studies

Accession	Collection Region	Latitude	Longitude	Altitude (m)	Accession	Collection Region	Latitude	Longitude	Altitude (m)
1	Amhara	93-71-75-N	39-41-95-E	3998	194	Oromia	09-33-20-N	37-29-18-E	2338
2	Amhara	65-23-30-N	57-15-13-E	3121	195	Oromia	10-24-41-N	38-44-13-E	2334
3	Amhara	64-66-20-N	57-15-63-E	3073	196	Oromia	10-39-41-N	36-59-58-E	2334
4	Amhara	10-35-04-N	37-46-41-E	2975	197	Oromia	09-33-57-N	37-30-14-E	2330
5	Amhara	12-46-00-N	37-36-00-E	2940	198	Oromia	09-28-24-N	37-28-24-E	2329
6	Amhara	93-42-84-N	39-40-65-E	2932	199	Oromia	09-29-55-N	37-26-56-E	2324
7	Amhara	93-41-32-N	39-40-04-E	2926	200	Oromia	09-29-55-N	37-26-56-E	2324
8	Amhara	09-14-00-N	38-53-00-E	2900	201	Oromia	09-29-59-N	37-26-50-E	2320
9	Amhara	10-28-28-N	37-57-06-E	2883	202	Oromia	09-42-13-N	38-23-03-E	2308
10	Amhara	94-15-50-N	39-37-54-E	2862	203	Oromia	09-23-50-N	37-25-15-E	2307
11	Amhara	11-08-39-N	37-25-34-E	2850	204	Oromia	09-24-11-N	37-25-24-E	2305
12	Amhara	11-44-45-N	38-05-33-E	2845	205	Oromia	10-45-59-N	37-11-21-E	2300
13	Amhara	11-45-31-N	38-05-31-E	2793	206	Oromia	09-27-30-N	37-25-32-E	2298
14	Amhara	10-31-25-N	37-41-60-E	2719	207	Oromia	09-27-33-N	37-25-57-E	2295
15	Amhara	38-02-00-N	11-51-00-E	2650	208	Oromia	09-25-34-N	37-25-34-E	2289
16	Amhara	10-52-00-N	38-28-00-E	2650	209	Oromia	10-12-41-N	37-30-29-E	2280
17	Amhara	11-49-00-N	37-50-00-E	2650	210	Oromia	09-24-12-N	37- 6-48-E	2275
18	Amhara	12-48-00-N	37-52-00-E	2650	211	Oromia	09-21-8 -N	37-7 -5 -E	2271
19	Amhara	10-58-02-N	36-57-12-E	2624	212	Oromia	09-21-9 -N	37- 7-21-E	2264
20	Amhara	38-18-00-N	10-38-00-E	2600	213	Oromia	08-47-00-N	36-39-00-E	2260
21	Amhara	10-28-23-N	37-57-33-E	2596	214	Oromia	09-26-00-N	41-21-00-E	2260
22	Amhara	12-23-39-N	37-41-57-E	2586	215	Oromia	09-03-00-N	40-50-00-E	2260
23	Amhara	10-34-04-N	38-09-01-E	2581	216	Oromia	08-37-00-N	38-12-00-E	2240
24	Amhara	10-34-04-N	38-09-01-E	2581	217	Oromia	10-03-13-N	36-51-46-E	2225
25	Amhara	12-23-41-N	37-41-57-E	2578	218	Oromia	12-33-09-N	37-06-07-E	2220

26	Amhara	10-49-19-N	37-03-00-E	2555	219	Oromia	07-15-00-N	40-20-00-E	2200
27	Amhara	10-26-17-N	37-56-41-E	2553	220	Oromia	08-58-00-N	37-30-00-E	2200
28	Amhara	11-34-21-N	38-08-44-E	2540	221	Oromia	11-34-21-N	38-08-44-E	2200
29	Amhara	10-11-00-N	38-09-00-E	2530	222	Oromia	09-05-00-N	40-21-00-E	2200
30	Amhara	10-24-45-N	37-49-46-E	2529	223	Oromia	08-58-00-N	37-30-00-E	2200
31	Amhara	10-24-41-N	38-44-13-E	2528	224	Oromia	09-01-00-N	38-20-00-E	2200
32	Amhara	11-36-44-N	37-56-46-E	2507	225	Oromia	09-24-00-N	42-17-00-E	2200
33	Amhara	11-24-14-N	37-50-40-E	2485	226	Oromia	10-42-16-N	37-06-21-E	2187
34	Amhara	10-55-16-N	37-45-24-E	2480	227	Oromia	09-05-59-N	36-35-09-E	2181
35	Amhara	09-43-00-N	37-46-00-E	2470	228	Oromia	09-10-00-N	41-32-00-E	2180
36	Amhara	10-16-59-N	37-48-23-E	2463	229	Oromia	10-42-43-N	37-05-22-E	2178
37	Amhara	10-52-01-N	37-00-20-E	2463	230	Oromia	11-57-48-N	36-59-02-E	2168
38	Amhara	10-15-28-N	38-07-53-E	2439	231	Oromia	11-55-07-N	37-53-27-E	2166
39	Amhara	11-53-32-N	37-58-10-E	2416	232	Oromia	10-04-31-N	36-28-01-E	2160
40	Amhara	10-24-00-N	37-23-00-E	2400	233	Oromia	08-55-00-N	36-28-00-E	2160
41	Amhara	11-09-05-N	36-51-54-E	2382	234	Oromia	09-50-09-N	36-27-27-E	2157
42	Amhara	11-23-38-N	37-54-02-E	2374	235	Oromia	09-31-40-N	34-31-37-E	2154
43	Amhara	10-04-44-N	37-44-54-E	2351	236	Oromia	09-45-53-N	36-38-54-E	2137
44	Amhara	10-04-44-N	37-49-54-E	2350	237	Oromia	09-50-28-N	36-23-16-E	2135
45	Amhara	10-13-00-N	37-45-00-E	2340	238	Oromia	08-48-15-N	36-40-27-E	2127
46	Amhara	11-39-03-N	37-53-00-E	2317	239	Oromia	09-56-38-N	03-10-42-E	2121
47	Amhara	11-55-42-N	37-57-08-E	2317	240	Oromia	09-52-06-N	36-41-15-E	2114
48	Amhara	11-10-10-N	36-51-53-E	2269	241	Oromia	09-01-00-N	36-25-00-E	2110
49	Amhara	11-43-00-N	37-41-00-E	2250	242	Oromia	08-48-34-N	34-48-18-E	2110
50	Amhara	10-16-38-N	37-34-03-E	2249	243	Oromia	12-06-56-N	36-59-03-E	2100
51	Amhara	10-17-06-N	37-34-49-E	2241	244	Oromia	09-30-00-N	42-00-00-E	2100
52	Amhara	10-14-49-N	37-32-25-E	2240	245	Oromia	09-00-00-N	38-09-00-E	2100
53	Amhara	11-22-44-N	37-25-01-E	2235	246	Oromia	08-34-24-N	34-43-59-E	2088
54	Amhara	10-19-29-N	37-37-35-E	2231	247	Oromia	08-45-00-N	36-38-00-E	2080

55	Amhara	10-19-29-N	37-37-35-E	2231	248	Oromia	08-37-00-N	38-13-00-E	2080
56	Amhara	10-15-47-N	37-33-04-E	2230	249	Oromia	10-49-19-N	37-03-00-E	2061
57	Amhara	10-18-44-N	37-29-37-E	2225	250	Oromia	09-02-07-N	36-24-10-E	2056
58	Amhara	10-19-25-N	37-38-49-E	2219	251	Oromia	08-51-10-N	35-06-36-E	2050
59	Amhara	37-18-00-N	11-07-00-E	2210	252	Oromia	08-58-00-N	38-01-00-E	2050
60	Amhara	11-11-32-N	36-51-28-E	2208	253	Oromia	08-26-00-N	34-51-00-E	2050
61	Amhara	10-16-11-N	37-26-15-E	2198	254	Oromia	08-09-00-N	39-21-00-E	2050
62	Amhara	10-16-25-N	37-26-20-E	2196	255	Oromia	08-58-00-N	38-01-00-E	2050
63	Amhara	10-18-13-N	37-29-33-E	2192	256	Oromia	09-56-55-N	36-30-52-E	2044
64	Amhara	10-16-28-N	37-26-24-E	2190	257	Oromia	10-42-53-N	37-08-21-E	2042
65	Amhara	10-25-18-N	37-22-12-E	2132	258	Oromia	07-52-00-N	37-25-00-E	2030
66	Amhara	11-18-87-N	36-46-94-E	2129	259	Oromia	08-54-16-N	36-56-42-E	2023
67	Amhara	10-12-41-N	37-30-29-E	2125	260	Oromia	08-38-53-N	34-42-12-E	2016
68	Amhara	11-55-07-N	37-53-27-E	2117	261	Oromia	08-58-00-N	37-52-00-E	2010
69	Amhara	10-33-46-N	37-27-57-E	2112	262	Oromia	08-58-00-N	37-52-00-E	2010
70	Amhara	12-23-95-N	37-01-11-E	2106	263	Oromia	08-58-00-N	37-52-00-E	2010
71	Amhara	10-42-43-N	37-05-22-E	2091	264	Oromia	09-02-00-N	36-41-00-E	1990
72	Amhara	11-41-01-N	37-37-27-E	2090	265	Oromia	10-52-01-N	37-00-20-E	1990
73	Amhara	10-31-29-N	37-31-16-E	2089	266	Oromia	09-11-26-N	36-59-04-E	1987
74	Amhara	10-31-22-N	37-31-21-E	2085	267	Oromia	08-47-19-N	36-23-57-E	1980
75	Amhara	10-42-16-N	37-06-21-E	2081	268	Oromia	09-13-34-N	36-52-49-E	1971
76	Amhara	10-32-74-N	37-26-30-E	2061	269	Oromia	09-55-24-N	36-47-33-E	1960
77	Amhara	10-22-07-N	37-22-20-E	2057	270	Oromia	11-53-32-N	37-58-10-E	1957
78	Amhara	10-50-37-N	36-34-60-E	2054	271	Oromia	08-32-00-N	34-46-00-E	1950
79	Amhara	11-28-00-N	37-10-00-E	2050	272	Oromia	08-32-00-N	34-46-00-E	1950
80	Amhara	10-23-08-N	37-22-18-E	2048	273	Oromia	08-32-00-N	34-46-00-E	1950
81	Amhara	10-23-10-N	37-22-08-E	2047	274	Oromia	08-59-00-N	37-48-00-E	1950
82	Amhara	10-39-26-N	36-59-22-E	2041	275	Oromia	11-11-32-N	36-51-28-E	1948
83	Amhara	10-39-41-N	36-59-58-E	2036	276	Oromia	08-54-28-N	36-32-22-E	1941

84	Amhara	10-45-59-N	37-11-21-E	2013	277	Oromia	09-58-12-N	36-18-37-E	1935
85	Amhara	11-34-00-N	37-23-00-E	2000	278	Oromia	09-23-58-N	35-43-27-E	1925
86	Amhara	10-42-53-N	37-08-21-E	1999	279	Oromia	11-33-13-N	37-18-26-E	1922
87	Amhara	12-33-09-N	37-06-07-E	1984	280	Oromia	07-05-00-N	37-57-00-E	1920
88	Amhara	11-20-00-N	36-58-00-E	1980	281	Oromia	08-38-27-N	34-50-37-E	1903
89	Amhara	10-39-00-N	37-24-00-E	1980	282	Oromia	09-56-38-N	03-10-42-E	1900
90	Amhara	12-05-00-N	37-44-00-E	1980	283	Oromia	11-10-10-N	36-51-53-E	1900
91	Amhara	08-42-23-N	36-26-43-E	1980	284	Oromia	09-00-02-N	35-03-27-E	1900
92	Amhara	11-25-00-N	37-12-00-E	1980	285	Oromia	08-50-00-N	40-36-00-E	1900
93	Amhara	11-20-00-N	36-58-00-E	1980	286	Oromia	08-50-00-N	40-36-00-E	1900
94	Amhara	11-20-00-N	36-58-00-E	1980	287	Oromia	08-37-00-N	39-45-00-E	1900
95	Amhara	11-27-44-N	37-16-82-E	1977	288	Oromia	09-15-00-N	42-25-00-E	1900
96	Amhara	11-50-00-N	37-34-00-E	1970	289	Oromia	09-14-19-N	37-02-32-E	1899
97	Amhara	12-26-00-N	37-30-00-E	1970	290	Oromia	08-35-00-N	35-48-48-E	1883
98	Amhara	12-00-00-N	37-20-00-E	1950	291	Oromia	08-29-00-N	34-48-00-E	1880
99	Amhara	12-10-32-N	37-41-28-E	1916	292	Oromia	08-26-00-N	36-20-00-E	1880
100	Amhara	12-21-50-N	37-15-07-E	1912	293	Oromia	08-25-44-N	35-52-48-E	1873
101	Amhara	12-08-56-N	37-44-20-E	1901	294	Oromia	08-55-22-N	36-35-27-E	1872
102	Amhara	37-44-00-N	12-05-00-E	1900	295	Oromia	09-09-12-N	36-28-03-E	1859
103	Amhara	12-19-42-N	37-04-41-E	1890	296	Oromia	09-20-00-N	41-54-00-E	1840
104	Amhara	12-06-56-N	36-59-03-E	1889	297	Oromia	08-18-00-N	39-20-00-E	1830
105	Amhara	12-12-47-N	37-38-11-E	1877	298	Oromia	08-50-09-N	36-57-03-E	1829
106	Amhara	06-56-00-N	37-51-00-E	1875	299	Oromia	09-11-47-N	35-45-15-E	1827
107	Amhara	11-33-13-N	37-18-26-E	1874	300	Oromia	07-58-00-N	36-29-00-E	1820
108	Amhara	12-22-43-N	37-17-26-E	1866	301	Oromia	07-58-00-N	36-29-00-E	1820
109	Amhara	11-57-48-N	36-59-02-E	1862	302	Oromia	08-20-08-N	35-44-07-E	1820
110	Amhara	12-22-00-N	37-20-00-E	1860	303	Oromia	09-12-00-N	35-44-00-E	1810
111	Amhara	11-55-07-N	37-53-27-E	1860	304	Oromia	08-24-00-N	39-15-00-E	1800
112	Amhara	11-55-00-N	37-22-00-E	1850	305	Oromia	09-03-00-N	36-42-00-E	1800

113	Amhara	11-57-00-N	37-37-00-E	1830	306	Oromia	09-10-00-N	35-41-00-E	1800
114	Amhara	10-40-41-N	37-22-04-E	1828	307	Oromia	08-23-04-N	35-39-02-E	1792
115	Amhara	10-40-41-N	37-22-04-E	1828	308	Oromia	09-14-46-N	34-32-51-E	1790
116	Amhara	11-28-43-N	37-23-33-E	1819	309	Oromia	09-06-00-N	37-00-00-E	1780
117	Amhara	10-38-43-N	37-16-57-E	1802	310	Oromia	09-03-00-N	36-44-00-E	1770
118	Amhara	11-30-07-N	37-29-11-E	1739	311	Oromia	08-53-00-N	40-33-00-E	1770
119	Amhara	09-48-12-N	35-00-43-E	1675	312	Oromia	09-34-17-N	35-23-35-E	1769
120	Amhara	10-15-47-N	37-33-04-E	1580	313	Oromia	08-10-00-N	35-33-00-E	1760
121	B/ Gumuz	10-35-47-N	35-45-15-E	2563	314	Oromia	08-20-00-N	35-43-00-E	1760
122	B/ Gumuz	10-35-05-N	35-45-27-E	2553	315	Oromia	09-10-52-N	35-37-42-E	1754
123	B/ Gumuz	10-35-04-N	35-46-22-E	2542	316	Oromia	07-55-00-N	37-25-00-E	1750
124	B/ Gumuz	10-34-41-N	35-43-01-E	2541	317	Oromia	08-32-00-N	35-40-00-E	1750
125	B/ Gumuz	10-34-31-N	35-42-05-E	2534	318	Oromia	08-52-00-N	40-42-00-E	1750
126	B/ Gumuz	10-34-11-N	05-42-43-E	2524	319	Oromia	08-57-59-N	34-59-04-E	1741
127	B/ Gumuz	10-33-50-N	35-40-39-E	2523	320	Oromia	09-03-00-N	36-52-00-E	1740
128	B/ Gumuz	10-34-36-N	35-41-25-E	2516	321	Oromia	08-32-00-N	35-40-00-E	1740
129	B/ Gumuz	10-39-23-N	35-41-44-E	2506	322	Oromia	09-03-00-N	36-52-00-E	1740
130	B/ Gumuz	10-35-54-N	35-39-46-E	2501	323	Oromia	09-03-00-N	36-52-00-E	1740
131	B/ Gumuz	10-40-15-N	35-42-01-E	2497	324	Oromia	09-09-19-N	35-36-58-E	1733
132	B/ Gumuz	10-39-10-N	35-40-21-E	2495	325	Oromia	09-00-10-N	36-50-40-E	1704
133	B/ Gumuz	10-39-57-N	35-41-08-E	2484	326	Oromia	09-03-00-N	37-10-00-E	1700
134	B/ Gumuz	10-00-34-N	34-36-28-E	1503	327	Oromia	09-17-00-N	42-10-00-E	1700
135	C. Ethiopia	07-21-00-N	37-46-00-E	2600	328	Oromia	08-32-20-N	35-38-28-E	1686
136	C. Ethiopia	37-59-00-N	07-40-00-E	2200	329	Oromia	08-38-00-N	34-57-00-E	1680
137	C. Ethiopia	37-57-00-N	07-11-00-E	2169	330	Oromia	08-12-00-N	35-34-00-E	1670
138	C. Ethiopia	07-05-00-N	37-57-00-E	1900	331	Oromia	08-33-17-N	35-38-21-E	1658
139	C. Ethiopia	07-22-60-N	36-13-03-E	1756	332	Oromia	08-56-13-N	37-03-55-E	1656
140	Harari	09-30-00-N	42-36-00-E	2100	333	Oromia	09- 6-59-N	35-37-44-E	1646
141	Harari	09-30-00-N	42-36-00-E	2100	334	Oromia	08-57-51-N	36-41-52-E	1641

142	Harari	09-15-00-N	42-05-00-E	1800	335	Oromia	09-48-12-N	35-00-43-E	1627
143	Harari	09-10-00-N	42-20-00-E	1700	336	Oromia	09-01-24-N	37-00-43-E	1623
144	Oromia	09-06-00-N	38-09-00-E	3000	337	Oromia	09-43-03-N	35-11-19-E	1613
145	Oromia	09-04-00-N	37-16-00-E	2820	338	Oromia	09-43-03-N	35-11-19-E	1600
146	Oromia	07-05-00-N	39-03-00-E	2650	339	Oromia	08-20-00-N	35-21-00-E	1600
147	Oromia	07-05-00-N	39-03-00-E	2650	340	Oromia	08-51-04-N	36-48-18-E	1582
148	Oromia	14-10-15-N	38-17-28-E	2620	341	Oromia	08-44-00-N	35-00-00-E	1550
149	Oromia	10-16-38-N	37-34-03-E	2608	342	Oromia	08-38-00-N	37-20-00-E	1530
150	Oromia	08-50-00-N	38-10-00-E	2600	343	Oromia	09-31-40-N	34-31-37-E	1526
151	Oromia	09-38-00-N	38-50-00-E	2600	344	Oromia	08-51-10-N	35-06-36-E	1513
152	Oromia	10-39-26-N	36-59-22-E	2574	345	Oromia	09-03-11-N	34-49-53-E	1512
153	Oromia	08-50-09-N	36-57-03-E	2560	346	Oromia	08-51-00-N	35-07-00-E	1500
154	Oromia	10-39-57-N	35-41-08-E	2550	347	S. Ethiopia	06-17-00-N	37-39-00-E	2750
155	Oromia	09-31-00-N	37-10-00-E	2540	348	S. Ethiopia	06-04-04-N	38-14-39-E	2549
156	Oromia	09-04-00-N	38-30-00-E	2540	349	S. Ethiopia	06-04-04-N	38-14-39-E	2549
157	Oromia	08-49-38-N	36-28-35-E	2524	350	S. Ethiopia	36-50-00-N	06-09-00-E	2380
158	Oromia	08-49-38-N	36-28-35-E	2524	351	S. Ethiopia	10-31-29-N	37-31-16-E	2089
159	Oromia	08-55-22-N	36-35-27-E	2504	352	S. Ethiopia	06-14-00-N	38-14-00-E	2000
160	Oromia	09-12-21-N	37-21-44-E	2501	353	S. Ethiopia	06-56-00-N	37-51-00-E	1900
161	Oromia	06-59-00-N	39-12-00-E	2500	354	S. Ethiopia	06-23-00-N	38-20-00-E	1853
162	Oromia	09-14-00-N	41-50-00-E	2500	355	S. Ethiopia	37-45-00-N	06-45-00-E	1850
163	Oromia	08-06-00-N	39-25-00-E	2480	356	S. Ethiopia	06-17-00-N	38-14-00-E	1820
164	Oromia	08-48-15-N	36-40-27-E	2467	357	S. Ethiopia	06-17-00-N	38-14-00-E	1820
165	Oromia	10-15-28-N	38-07-53-E	2452	358	S. Ethiopia	06-30-00-N	38-15-00-E	1800
166	Oromia	12-19-42-N	37-04-41-E	2450	359	S. Ethiopia	05-55-00-N	37-30-00-E	1800
167	Oromia	10-31-29-N	37-31-16-E	2446	360	S. Ethiopia	10-18-44-N	37-29-37-E	1580
168	Oromia	07-03-00-N	40-01-00-E	2430	361	SW Ethiopia	07-05-72-N	37-02-51-E	2643
169	Oromia	08-44-00-N	36-40-00-E	2420	362	SW Ethiopia	07-05-47-N	37-02-38-E	2635
170	Oromia	08-48-00-N	37-40-00-E	2410	363	SW Ethiopia	07-05-17-N	37-02-91-E	2537

171	Oromia	08-47-19-N	36-23-57-E	2403	364	SW Ethiopia	07-16-66-N	35-54-26-E	1981
172	Oromia	10-01-29-N	44-55-92-E	2400	365	SW Ethiopia	07-17-40-N	35-54-23-E	1975
173	Oromia	09-33-00-N	37-03-00-E	2400	366	SW Ethiopia	07-16-03-N	35-55-88-E	1920
174	Oromia	09-33-00-N	37-03-00-E	2400	367	SW Ethiopia	07-16-00-N	36-14-00-E	1800
175	Oromia	09-33-00-N	37-03-00-E	2400	368	SW Ethiopia	07-22-16-N	36-15-83-E	1760
176	Oromia	10-01-29-N	44-55-95-E	2399	369	SW Ethiopia	07-16-70-N	35-23-95-E	1682
177	Oromia	10-01-29-N	44-55-95-E	2399	370	Tigray	14-13-54-N	39-10-44-E	2506
178	Oromia	09-29-49-N	37-26-14-E	2396	371	Tigray	07-22-60-N	36-13-03-E	2500
179	Oromia	08-54-42-N	36-29-09-E	2395	372	Tigray	13-57-13-N	39-36-26-E	2460
180	Oromia	39-16-07-N	37-06-46-E	2394	373	Tigray	13-36-52-N	39-12-02-E	2366
181	Oromia	10-01-29-N	44-55-98-E	2392	374	Tigray	14-07-00-N	38-45-00-E	2130
182	Oromia	10-15-20-N	37-32-46-E	2392	375	Tigray	14-17-16-N	39-01-13-E	2110
183	Oromia	10-01-29-N	44-55-98-E	2392	376	Tigray	14-12-00-N	38-56-00-E	2110
184	Oromia	07-22-00-N	40-12-00-E	2390	377	Tigray	14-12-00-N	38-56-00-E	2110
185	Oromia	10-40-41-N	37-22-04-E	2383	378	Tigray	14-04-26-N	38-33-45-E	2071
186	Oromia	07-12-00-N	39-11-00-E	2380	379	Tigray	13-55-35-S	39-22-46-E	2051
187	Oromia	08-50-42-N	36-29-08-E	2377	380	Tigray	13-49-09-N	39-36-36-S	2033
188	Oromia	10-19-25-N	37-38-49-E	2361	381	Tigray	13-29-00-N	39-30-00-E	2000
189	Oromia	09-16-21-N	37-11-15-E	2350	382	Tigray	14-07-35-N	39-00-13-E	1993
190	Oromia	09-23-00-N	37-00-00-E	2350	383	Tigray	14-10-15-N	38-17-28-E	1932
191	Oromia	08-49-38-N	36-28-35-E	2342	384	Tigray	13-32-47-N	39-03-57-E	1702
192	Oromia	08-00-00-N	39-12-00-E	2340	385	Tigray	14-01-00-N	38-44-00-E	1700
193	Oromia	09-28-27-N	37-28-30-E	2338	386	Tigray	13-54-55-N	38-36-26-E	1687

Appendix Table 3.1. List of *B. carinata* accessions used in the study and some morphological and seed oil composition attributes

Accession No	Name	Collection Region	SY (t/ha)	OC (%)	EA (%)	Accession No	Name	Collection Region	Seed Yield (t/ha)	Oil content (%)	Erucic acid content (%)
1	35120	Amhara	1.91	43.52	42.44	95	208601	Oromia	2.26	44.76	42.28
2	20005	Amhara	1.62	45.41	43.26	96	17579	Oromia	2.04	44.33	44.83
3	20106	Amhara	1.58	41.75	52.07	97	24469	Oromia	1.55	43.79	53.66
4	200011	Amhara	1.49	45.48	48.17	98	21320	Oromia	1.65	45.91	44.65
5	20017	Amhara	1.62	41.51	46.83	99	28349	Oromia	1.97	46.51	40.00
6	20144	Amhara	1.93	41.60	52.77	100	21320	Oromia	1.66	44.16	52.31
7	21017	Amhara	1.81	41.53	48.76	101	20127	Oromia	1.62	46.46	34.29
8	20023	Amhara	1.34	41.56	54.34	102	215793	Oromia	1.76	44.71	49.09
9	21012	Amhara	2.04	45.11	46.87	103	21349	Oromia	1.49	43.71	44.12
10	1004	Amhara	1.79	45.07	48.11	104	24468	Oromia	1.50	44.95	53.05
11	20100	Amhara	1.42	41.14	53.91	105	21345	Oromia	1.61	46.25	49.64
12	9664	Amhara	1.95	46.14	53.06	106	24476	Oromia	1.88	42.27	49.47
13	18081	Amhara	1.50	42.26	50.66	107	21322	Oromia	2.03	41.24	51.36
14	208406	Amhara	1.84	46.72	51.82	108	208590	Oromia	1.55	41.81	51.62
15	35130	Amhara	1.58	43.36	45.89	109	21318	Oromia	1.51	44.61	44.20
16	21384	Amhara	1.54	43.18	46.70	110	17575	Oromia	1.82	45.00	51.82
17	18044	Amhara	1.93	43.03	50.63	111	28344	Oromia	1.27	44.26	52.32
18	18041	Amhara	2.06	46.63	48.83	112	208804	Oromia	1.71	46.52	47.28
19	19962	Amhara	1.67	41.28	54.83	113	21321	Oromia	1.42	38.15	52.41
20	35131	Amhara	2.18	41.30	52.73	114	207918	Oromia	2.21	41.60	50.38
21	208405	Amhara	1.83	46.67	40.16	115	207918	Oromia	1.62	46.05	51.47
22	18079	Amhara	1.95	43.98	52.25	116	21358	Oromia	2.18	41.38	48.92
23	18080	Amhara	1.98	43.93	54.26	117	21352	Oromia	1.43	43.91	35.40
24	26687	Amhara	1.88	43.55	51.74	118	24467	Oromia	1.85	46.39	53.02
25	26685	Amhara	1.58	44.23	50.62	119	17583	Oromia	1.41	41.93	54.51
26	26681	Amhara	1.60	46.85	51.96	120	208807	Oromia	1.78	42.52	44.65
27	35116	Amhara	2.01	44.10	45.26	121	208608	Oromia	1.36	38.11	44.95

28	9665	Amhara	1.52	46.08	55.69	122	17548	Oromia	1.88	46.18	51.28
29	18042	Amhara	1.81	44.58	52.47	123	21358	Oromia	1.54	44.07	48.20
30	18088	Amhara	1.80	46.55	47.17	124	28346	Oromia	1.67	41.34	51.80
31	26688	Amhara	1.70	42.67	50.48	125	28347	Oromia	1.44	46.91	53.62
32	26692	Amhara	1.75	42.76	47.46	126	17585	Oromia	1.41	41.16	53.76
33	18073	Amhara	1.70	44.50	51.27	127	18071	Oromia	2.11	43.11	49.62
34	18085	Amhara	1.41	41.91	51.85	128	17568	Oromia	1.86	41.51	46.83
35	26675	Amhara	1.57	39.50	52.51	129	20030	Oromia	1.47	41.22	50.84
36	26673	Amhara	1.59	42.96	53.63	130	20030	Oromia	1.95	41.64	51.92
37	208416	Amhara	2.08	45.99	45.69	131	208353	Oromia	1.91	41.68	44.41
38	18071	Amhara	1.92	44.83	48.08	132	208603	Oromia	2.07	45.46	47.25
39	35122	Amhara	1.95	42.92	52.67	133	17565	Oromia	1.70	46.23	50.87
40	215824	Amhara	1.61	44.81	51.27	134	208717	Oromia	1.91	42.83	50.95
41	26693	Amhara	1.76	43.13	50.48	135	245037	Oromia	2.12	45.30	46.64
42	35126	Amhara	1.42	42.31	48.56	136	24471	Oromia	2.01	44.97	44.44
43	208423	Amhara	1.89	44.78	50.69	137	17550	Oromia	1.39	43.40	50.04
44	18093	Amhara	1.40	43.22	45.86	138	20223	Oromia	1.44	43.54	51.92
45	18075	Amhara	1.98	44.43	49.47	139	245010	Oromia	1.92	42.44	45.54
46	26696	Amhara	1.51	41.41	37.17	140	215793	Oromia	1.95	44.65	50.36
47	18082	Amhara	2.03	43.59	42.73	141	215792	Oromia	1.97	42.87	49.42
48	35121	Amhara	1.66	41.98	50.02	142	20066	Oromia	1.57	44.12	43.70
49	18076	Amhara	1.84	44.05	51.29	143	245014	Oromia	1.56	45.36	53.83
50	24122	Amhara	1.69	42.12	49.09	144	17559	Oromia	1.91	46.71	44.12
51	24121	Amhara	1.27	43.61	45.63	145	17578	Oromia	1.95	45.30	46.64
52	26697	Amhara	1.84	44.25	47.92	146	203232	Oromia	1.71	41.36	44.38
53	208407	Amhara	1.58	46.85	52.23	147	17576	Oromia	2.00	39.32	55.42
54	35127	Amhara	1.48	42.50	54.46	148	21323	Oromia	1.50	44.03	46.57
55	35128	Amhara	1.71	42.68	50.65	149	245029	Oromia	1.73	44.62	47.54
56	26672	Amhara	1.72	41.14	52.03	150	20134	Oromia	2.23	45.68	48.62
57	26684	Amhara	1.85	44.53	52.08	151	21358	Oromia	2.15	45.47	47.84
58	23603	B/Gumu	1.99	43.63	49.21	152	21357	Oromia	2.12	45.42	47.81
59	23597	B/Gumu	1.76	41.21	50.41	153	17563	Oromia	1.48	44.97	44.44

60	23592	B/Gumu	1.48	44.79	46.69	154	17580	Oromia	1.82	42.08	44.87
61	23593	B/Gumu	1.52	44.70	44.12	155	21371	Oromia	1.37	41.14	51.06
62	23595	B/Gumu	1.57	44.44	51.69	156	245031	Oromia	1.49	45.59	36.77
63	23596	B/Gumu	2.11	44.28	50.49	157	17555	Oromia	1.74	42.00	52.77
64	24202	B/Gumu	1.55	42.85	43.67	158	20134	Oromia	1.89	42.70	50.93
65	23589	B/Gumuz	1.94	46.77	49.27	159	21340	Oromia	1.64	41.76	50.39
66	23590	B/Gumu	2.47	46.83	41.59	160	21006	Oromia	1.33	40.16	51.08
67	23591	B/Gumu	1.64	46.87	50.41	161	20134	Oromia	2.06	45.16	50.00
68	18086	C Ethiopia	1.59	43.47	50.77	162	17546	Oromia	1.42	41.66	50.23
69	21361	C Ethiopia	1.71	45.61	50.77	163	245030	Oromia	1.93	44.23	50.47
70	208860	C Ethiopia	1.95	43.89	46.73	164	21327	Oromia	2.02	45.77	50.30
71	208602	Harari	1.76	46.31	49.35	165	20049	Oromia	1.99	42.75	53.16
72	208598	Harari	2.12	42.04	52.89	166	17561	Oromia	1.50	45.46	47.25
73	230791	Harari	1.68	44.92	54.62	167	201065	Oromia	1.67	46.75	51.56
74	208959	Oromia	1.56	44.84	36.31	168	208599	Oromia	1.90	41.28	54.83
75	231336	Oromia	1.56	42.22	44.93	169	20055	Oromia	1.50	44.41	41.20
76	Tesfa	Oromia	2.40	37.89	49.36	170	17557	Oromia	1.69	45.19	52.50
77	Holeta-1	Oromia	2.50	43.46	50.96	171	17592	Oromia	1.47	44.32	48.21
78	Derash	Oromia	2.40	43.44	47.38	172	20076	S Ethiopia	2.05	38.65	38.99
79	Yellow Dodola	Oromia	2.54	43.43	50.16	173	21384	S Ethiopia	1.54	41.72	51.34
80	S-57	Oromia	2.31	46.99	34.65	174	215561	S Ethiopia	1.78	43.18	46.70
81	208586	Oromia	1.49	44.74	44.93	175	245023	S Ethiopia	1.54	44.4	48.83
82	207919	Oromia	2.16	41.49	45.57	176	208860	S Ethiopia	1.38	46.79	46.29
83	17582	Oromia	1.83	42.17	50.42	177	20905	SW Ethiopia	1.91	45.86	55.58
84	207923	Oromia	1.42	45.66	46.92	178	20907	SW Ethiopia	2.05	46.75	51.56
85	17572	Oromia	1.85	39.13	48.81	179	20916	SW Ethiopia	1.54	46.63	48.83
86	17577	Oromia	2.02	43.87	54.26	180	26132	Tigray	1.52	46.21	52.23
87	17549	Oromia	2.07	43.68	53.36	181	26128	Tigray	1.61	42.01	47.65
88	24470	Oromia	1.94	46.02	50.20	182	20077	Tigray	1.77	42.33	49.51
89	28351	Oromia	2.08	41.96	52.54	183	26125	Tigray	2.47	41.80	49.72
90	28351	Oromia	1.95	44.45	44.92	184	26124	Tigray	1.53	42.89	50.44
91	21346	Oromia	1.77	41.02	46.62	185	219786	Tigray	2.17	42.57	52.23

92	21338	Oromia	2.03	39.88	47.99	186	26131	Tigray	2.06	42.41	49.88
93	28349	Oromia	1.71	44.69	47.43	187	26121	Tigray	1.83	43.03	50.63
94	20127	Oromia	1.49	43.52	42.44	188	221694	Tigray	1.547	42.24	50.20

Appendix Table 3.2: Distribution of 3,793 DArTseq SNPs across *B. carinata* chromosomes, including density per chromosome, polymorphic information content (PIC), minor allele frequency (MAF), and major allele frequency (MaF)

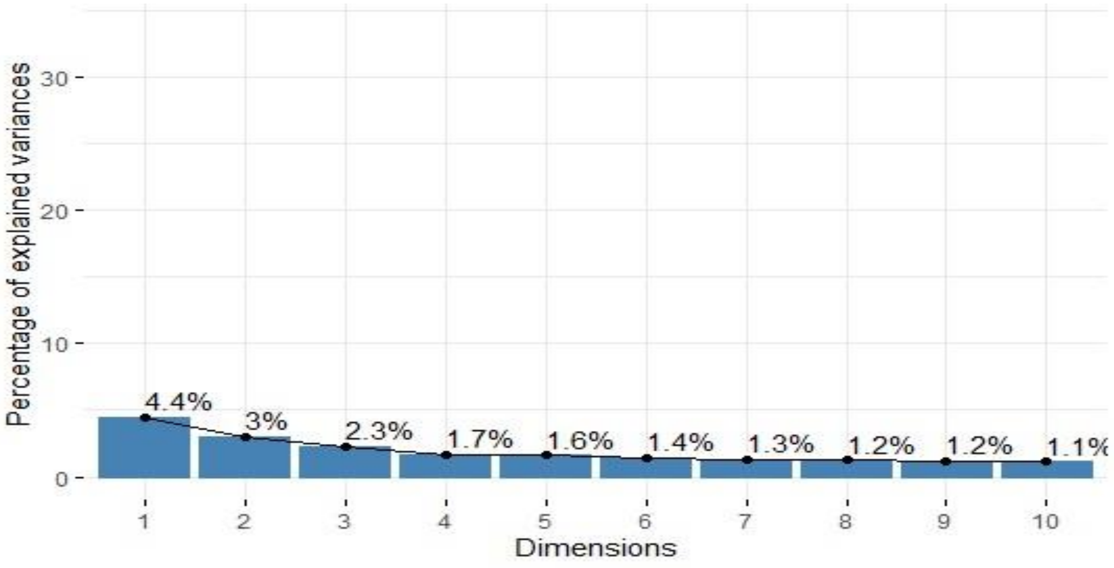
Chromosome	No. SNPs	Chr. length (Mb)	SNPs Density/Mb	PIC	MAF	MaF
B1	376	77.380	4.859	0.134	0.098	0.115
B2	213	61.106	3.486	0.131	0.107	0.123
B3	209	58.891	3.550	0.140	0.108	0.125
B4	225	58.210	3.865	0.147	0.116	0.15
B5	171	57.410	2.979	0.137	0.104	0.117
B6	183	55.502	3.297	0.127	0.091	0.108
B7	209	53.286	3.922	0.111	0.078	0.094
B8	136	45.607	2.982	0.113	0.079	0.098
Sub-genome B	**1722**	**58.424**	**3.618**	**0.13**	**0.098**	**0.116**
C 1	159	71.916	2.211	0.135	0.102	0.112
C 2	189	67.422	2.803	0.113	0.079	0.091
C 3	111	66.419	1.671	0.117	0.084	0.096
C 4	110	65.755	1.673	0.133	0.115	0.151
C 5	178	65.555	2.715	0.114	0.085	0.097
C 6	199	60.001	3.317	0.134	0.100	0.127
C 7	151	56.612	2.667	0.141	0.113	0.131
C 8	107	53.443	2.002	0.133	0.101	0.113
C9	103	49.557	2.078	0.119	0.102	0.116
Sub-genome C	**1307**	**61.853**	**2.349**	**0.127**	**0.098**	**0.115**
Contigs			764			
Whole genome	**3793**	**8.4**	**2.984**	**0.128**	**0.098**	**0.116**

GD = Gene diversity, Ho = Observed heterozygosity, PIC = Polymorphic information content, MAF = Minor allele frequency, MaF = Major allele frequency.

Appendix Table 3.4: Percentage of variation explained by the first five principal components (PCs) and their corresponding eigenvalues for the 188 *B. carinata* accessions

PC	Eigenvalue	Variance (%)	Cumulative variance (%)
PCA 1	49.64	4.42	4.42
PCA 2	33.98	3.03	7.44
PCA 3	25.49	2.27	9.71
PCA 4	18.83	1.68	11.39
PCA 5	17.90	1.59	12.98

Appendix Figure-4.1 Bar plot from structure analysis (K = 2) showing two distinct groups among the 188 *B. carinata* accessions: population-1 (red) and population-2 (green). Each bar represents an individual accession, with numbers under each bar corresponds to those accession numbers in Appendix Table 2.



Appendix Figure-4.2 Scree plot illustrating the principal components for the 188 *B. carinata* accessions.