Pre-breeding Sesame (*Sesamum indicum* L.) for Improved Yield, and Oil Quality and Quantity in Ethiopia

By

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Thesis Abstract

Sesame (*Sesamum indicum* L.; 2n = 2x = 26) is a multi-purpose industrial oilseed crop serving the food, feed, and cosmetic industries globally. Sesame is Ethiopia's most valuable export crop after coffee (*Coffea arabica* L.), contributing to socio-economic development. However, the productivity of the crop is low (<0.6 ton ha^{-1}) and stagnant in Ethiopia and other major sesame growing regions in sub-Saharan Africa due to a multitude of production constraints. The low yield of sesame is attributable to lack of high-yielding and well-adapted varieties, with less capsule shattering; resistant/tolerant to biotic and abiotic stresses; a lack of modern crop production technologies and well developed infrastructure. Sesame remains a largely under-researched and underutilized crop in Ethiopia despite its economic value in the local, regional and international trades. There is a need for a dedicated sesame genetic improvement programme to develop and deploy new improved varieties with farmer- and market-preferred traits. Therefore, the specific objectives of this study were: i) to document sesame production opportunities and constraints and farmer-and market-preferred varieties and traits in eastern and southwestern Ethiopia as a guide for breeding; ii) to determine the variance components, broad-sense heritability (h^2b) and association of seed and oil yield-related traits in Ethiopian sesame germplasm for effective breeding; iii) to determine the extent of genetic variation among 100 diverse sesame germplasm collections of Ethiopia using phenotypic traits and simple sequence repeat (SSR) markers and select distinct and contrasting genotypes for breeding and iv) to determine the genetic diversity and relationships among Ethiopia's sesame germplasm collections using seed oil content and fatty acid compositions and diagnostic SSR markers and select genetically unique and promising parental lines for breeding. Different but complementary research activities were conducted to attain the objectives.

The first study was conducted using a participatory rural appraisal (PRA) involving 160 farmers in two selected sesame growing regions and four districts in Ethiopia. A considerable proportion of the respondent farmers (56.0%) reported cultivating sesame using seeds of unknown varieties often sourced from the informal seed sector. The most important constraints to sesame production in the study areas were lack of access to improved seeds (reported by 83.0% of respondents), low yield potential of the existing varieties (73.8%), diseases (69.4%), and low market price (68.8%). These constraints were attributed to the lack of a dedicated breeding programme, formal seed sector, strong extension services, and well-developed pre-and post-harvest infrastructures. The most important market-preferred traits of sesame included true-to-type seed, white seed colour, and high seed oil content. Reasonable market price, resistance to crop diseases, drought tolerance, resistance to crop insect pests, higher seed yield, higher thousand-seed weight, higher oil content, white seed colour,

early maturity, and good oil qualities such as aroma and taste were the vital farmer-preferred attributes in order of significance. Hence, these traits should be integrated in current and future sesame breeding programs.

The second study evaluated 100 sesame germplasm under field conditions at two locations using a 10 x 10 lattice design with two replications. The findings revealed a higher genotypic coefficient of variation and h^2b values for the number of primary branches (NPB), number of secondary branches (NSB), thousand seed weight (TSW), seed yield per hectare (SYH) and oil yield per hectare (OYH), suggesting that high genetic gains can be achieved through selection. Higher direct effects of OYH and number of seeds per capsule (NSPC) were recorded affecting SYH, while SYH, number of capsules per plant (NCPP) and TSW had a higher direct effect on OYH. Genotypes Hirhir Kebabo Hairless-9, Setit-3, Orofalc ACC-2, Hirhir Humera Sel-6, Setit-1 and ACC-NS-007(2) were selected for further breeding based on their high seed yield, oil content and oil yield.

In the third part of the study, 100 sesame entries were field evaluated at two locations in Ethiopia for agro-morphological traits and seed oil content using a 10 × 10 lattice design with two replications. Also, test genotypes were profiled using 27 polymorphic SSR markers at the Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences. Analysis of variance revealed significant ($p \leq 0.05$) entry by environment interaction for plant height, internode length, number of secondary branches, and grain yield. Genotypes such as Hirhir Kebabo Hairless-9, Setit-3, Orofalc ACC-2, Hirhir Humera Sel-6, ABX = 2-01-2, and Setit-1 recorded grain yield of >0.73 ton ha^{-1}. Grain yield had positive and significant ($p < 0.01$) associations with oil yield ($r = 0.99$), which is useful for simultaneous selection. Moderate gene diversity and polymorphic information content values of 0.30 and 0.25 were recorded based on SSR analysis, respectively. The genotypes were separated into two and four major distinct groups based on cluster and population structure analyses, respectively, thus enabling selection and subsequent crossing to develop breeding populations for cultivar development. Based on phenotypic and genomic divergence, the following superior and complementary genotypes were selected: Hirhir Humera Sel-6, Setit-3, Hirhir Kebabo Hairless Sel-4, Hirhir Nigara 1st Sel-1, Humera-1 and Hirhir Kebabo Early Sel-1 (from cluster II-a), Hirhir kebabo hairless-9, NN-0029(2), NN0068-2 and Bawnji Fiyel Kolet, (from cluster II-b). The selected genotypes will serve as parents for sesame breeding program in Ethiopia.

In the fourth part of the study, the contents of the seed oil and fatty acids of 100 sesame lines were determined using near-infrared reflectance spectrometry (NIRS). Twenty-seven polymorphic SSR markers were used to assess the genetic profile of the test lines and complement the seed oil and fatty acid contents. The oil ranged from 44.30 to 55.60%, with a mean of 49.84% followed by the oleic

acid (36.70 to 48.80%, with a mean of 42.90%) and linoleic acid (36.60 to 47.10%, mean 41.70%). The SSR markers resolved the test genotypes into two major clusters, each with two sub-clusters. Lines such as: Hirhir Kebabo Hairless Sel-6 (from sub-cluster I-b), Hirhir Humera Sel-8 and NN0058-2 (sub-cluster II-a) and Bawnji Fiyel Kolet (sub-cluster II-b) were identified for sesame breeding programs based on higher oil content and desirable fatty acid compositions and SSR profiles.

Overall, the present study appraised the current farmers' major production constraints and farmer- and market-preferred varieties and traits of sesame as a guide for large-scale production and breeding. Additionally, the study identified unique sesame genetic resources with high-yield and yield component traits, quantity and quality seed oil content and genetic profiles for breeding programs in Ethiopia and elsewhere.

Declaration

I, Desawi Hdru Teklu, declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original research.

2. This thesis has not been submitted for any degree or examination at any other University.

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

a. Their words have been re-written, but the general information attributed to them has been referenced.

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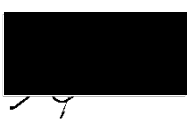
5. This thesis does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the thesis and in the references sections.

Signed:

Desawi Hdru Teklu

As the candidate's supervisor, I agree to the submission of this dissertation:



Prof. Hussein Shimelis (Supervisor)

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Dedication

This thesis work is dedicated to my

late-Grandmama, Mrs. *Letebirhan Desta (Ashetahaney)*

wife, *Bisrat Mekonnen*,

daughter, *Yohanan Desawi*

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Publications Pertaining to this Thesis

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Sesame (*Sesamum indicum* L.) improvement for seed and oil yields and fatty acid composition: progress and outlook: A review. Under review in the Journal of Food Composition and Analysis

Chapter Two

Teklu, D.H.; Shimelis, H.; Tesfaye, A.; Abady, S. Appraisal of the Sesame Production Opportunities and Constraints, and Farmer-Preferred Varieties and Traits, in Eastern and Southwestern Ethiopia. Sustainability 2021, 13, 11202. https://doi.org/ 10.3390/su132011202

Chapter Three

Teklu DH, Shimelis H, Tesfaye A, Mashilo J. Genetic diversity and association of yield-related traits in sesame. Plant Breeding 2021; 140:331–341. https://doi.org/10.1111/pbr.12911

Chapter Four

Teklu, D.H.; Shimelis, H.; Tesfaye, A.; Mashilo, J.; Zhang, X.; Zhang, Y.; Dossa, K.; Shayanowako, A.I.T. Genetic Variability and Population Structure of Ethiopian Sesame (*Sesamum indicum* L.) Germplasm Assessed through Phenotypic Traits and Simple Sequence Repeats Markers. Plants 2021, 10, 1129. https://doi.org/10.3390/plants10061129

Chapter Five

Teklu, D.H.; Shimelis, H.; Tesfaye, A.; Mashilo, J. Analyses of Genetic Diversity and Population Structure of Sesame (*Sesamum indicum* L.) Germplasm Collections through Seed Oil and Fatty Acid Compositions and SSR markers. Journal of Food Composition and Analysis. https://doi.org/10.1016/j.jfca.2022.104545

Thesis introduction

Background

Sesame (*Sesamum indicum* L) is one of the oldest oilseed crops cultivated for food, feed, and cosmetics. Sesame seed has the highest oil content (60%) when compared to other oilseed crops such as soybean (~20%), rapeseed (~40%), sunflower (~45%), and groundnut (45-56%) (De la Vega and Hall 2002; Zheljazkov et al. 2008; Wei et al. 2013; Gulluoglu et al. 2016; Dossa et al. 2018). There are several human health benefits of the consumption of sesame oil. The seed has essential nutritional benefits, including antioxidant, antiaging, antihypertensive, anticancer, and cholesterol-lowering properties. The seed oil is a rich source of protein (~24%), carbohydrate (~13.5%), vitamins (e.g., A and E), lignans (sesamin and sesamolin), and lipids (Were et al. 2006; Anastasi et al. 2017; Gharby et al. 2017; Dossa et al. 2018). The sesame oil seedcake obtained after oil extraction contains about 32% crude protein (CP) and 8-10% oil, an excellent feed for livestock and poultry (Yasothai et al. 2014). The sesame stalk is used for animal feed, manufacturing soap, compost, and potash, a cooking ingredient widely used in West African countries (Dosaa et al. 2017). The aforementioned and other benefits make sesame a highly valued crop in local, regional and international markets (Anilakumar et al. 2010).

Sesame is cultivated on about 14.24 million hectares with an annual production of ≈7.23 million tons worldwide (FAOSTAT 2020). Sudan is the largest sesame producing country with a total annual grain production of 1,525,104.00 tons, followed by China (896,630.00 tons), Myanmar (740,000.00 tons), the United Republic of Tanzania (710,000.00 tons), India (658,000.00 tons), Nigeria (490,000.00 tons), , Burkina Faso (270,000.00 tons) and Ethiopia (260,258.00 tons) (FAOSTAT 2020). According to FAOSTAT (2020), Africa produced about 4,080,917.00 tons of sesame in 2020, of which Ethiopia's share was 260,258.00 tons (6.38%). Globally, 2,413,114.00 tons of sesame grain was traded with a monetary value of 3.2 trillion USD in 2020 (FAOSTAT 2020). In 2020 sub-Saharan African countries exported about 1,515,853.00 tons of unprocessed sesame with a cash value of 1.7 trillion USD (FAOSTAT 2020).

Sesame is Ethiopia's most valuable export crop after coffee (*Coffea arabica* L.). In 2020, Ethiopia's sesame export share was 9.45% of global exports, valuing 298.3 million USD (FAOSTAT 2020). In the country, sesame is widely produced for household food and as a source of cash (Teklu et al. 2021a). It is predominantly grown by smallholders (95.5%) and medium-to-large commercial farmers (0.5%) under rainfed conditions. Sesame production is widely practised in the lowland areas of the country

owing to drought, and heat stresses tolerance of the crop. According to the Ethiopian Central Statistical Agency (CSA 2020) in the 2019/2020 production seasons, the total area and volume of sesame production under medium-to-large commercial farming conditions was the highest in the following regions: Tigray (56.42%), Amhara (32.03%), Benishangul-Gumuz (7.25%), and Oromia (3.17%). The total area and volume of production under smallholder farming systems was the highest in Amhara (51.82%), Tigray (30.88%), Oromia (9.41%), and Benishangul-Gumuz (7.34%) regions of Ethiopia (CSA 2020).

Sesame remains a largely under-researched and underutilized crop in Ethiopia despite its economic values (Teklu et al. 2021b). The productivity of sesame is low (≤0.7 t/ha) (CSA 2020) and remains stagnant in Ethiopia because of many production constraints. The low yield of sesame is attributable to lack of high-yielding and well-adapted varieties, with less capsule shattering, resistant/tolerant to biotic and abiotic stresses, and lack of modern production technologies such as optimal agronomic management practices, row planters, harvesters, and storage facilities (Were et al. 2006; Nyongesa et al. 2013; Woldesenbet et al. 2015, Anyanga et al. 2017; Dossa et al. 2017; Teklu et al. 2021a). These production constraints have yet to be systematically studied, prioritized, and documented in Ethiopia to guide the research and development of the crop. This will serve as market research to guide variety design and development and develop a successful marketing strategy.

The Ethiopian Agricultural Research Institute (EIAR) and other regional research centres are involved in sesame improvement in Ethiopia. During the last 46 years, a total of 32 improved sesame varieties were developed and released by the EIAR mainly through mass selection from among the local germplasm collections, introduction and crossing of selected parents (MoA 2019). The sesame varieties designated as Humera-1 and Setit-1 were released by the Humera Agricultural Research Centre (HuARC) in 2010. These varieties are widely grown by farmers for their early maturity, high yield performance, and broad adaptability. The yield performance of these varieties is low (<1.00 ton/ha), below the reportedly attainable yields of the crop at 3.29 and 2.38 tons/ha, such as in Lebanon and Jordan, respectively (FAOSTAT 2020). A study showed that lack of access to improved seeds and low yield from cultivating the existing varieties were the most important production constraints attributed to the lack of dedicated breeding program, formal seed sector, good extension services, and well developed pre-and post-harvest infrastructures in the country (Teklu et al. 2021a). So far, the local sesame breeding programme has mainly focused on characterization and mass selection of landrace collections for desirable traits for large-scale production, marketing, and further

breeding (Teklu et al. 2021b). Consequently, farmers mostly use landrace varieties of the crop that are inherently low yielders and prone to capsule shattering, leading to reduced productivity and low income. However, landraces are highly valued for having farmer-preferred attributes such as unique taste, aroma, and adaptation to grow under low-input farming systems and marginal agricultural lands.

Rationale of the study

Ethiopia is the centre of genetic diversity of sesame (Bedigian 1981; Seegeler 1983). The Ethiopian Biodiversity Institute (EBI) maintains about 5000 accessions of sesame germplasm collections (Teklu et al. 2021b). The sesame germplasm, including landraces and exotic introductions, can be an ideal source of genetic variation to initiate sesame pre-breeding program. These genetic resources need to be systematically evaluated based on seed yield and yield-related traits, oil quantity and quality and high-throughput molecular markers to select contrasting parents for hybridization or to develop high-performing varieties or direct production. This will allow sustainable production and meet the marketplace standard requirements. Sesame production in Ethiopia relies on a limited number of genetically unimproved landrace varieties selected by farmers. Landraces or traditional varieties have low yields but possess intrinsic seed oil quality characteristics, such as unique aroma and taste useful for breeding (Teklu et al. 2021b). Previous studies have reported considerable phenotypic variation for agronomic and quality traits in Ethiopia's sesame genetic resources (Gidey et al. 2012; Teklu et al. 2014; Hika et al. 2014;2015). However, earlier studies did not fully represent the landrace collections from various parts of Ethiopia. Hence, a comprehensive assessment of the genetic diversity present in the Ethiopian sesame genetic resources is needed using a relatively more significant number of accessions representing the diverse germplasm resources sampled from various regions through phenotypic traits and molecular markers.

Knowledge of the genetic profiles of diverse germplasm collections of sesame using yield components, seed oil and fatty acid contents, and molecular markers is a prerequisite to developing market-preferred cultivars with higher quantity and quality oil. Additionally, in Ethiopia, no recent study has documented farmers' perceptions of the production constraints on sesame and the preferred traits that farmers, markets, and the value chain require in a new sesame variety. Selection and identification of desirable sesame genotypes is fundamental for breeding, genetic analyses, gene discovery, and developing high-performing and farmer-preferred varieties.

Overall research goal

The overall goal of this study was to develop high-yielding sesame genotypes that have local adaptation, high oil quality and quantity, and unique genetic profiles.

Specific objectives

The specific objectives of the study were:

i) To document sesame production opportunities and constraints and farmer- and market-preferred varieties and traits in eastern and southwestern Ethiopia as a guide for large-scale production and breeding.
ii) To determine the variance components, heritability and association of seed and oil yield-related traits in Ethiopian sesame germplasm for effective breeding.
iii) To determine the extent of genetic variation among 100 diverse sesame germplasm collections of Ethiopia using phenotypic traits and simple sequence repeat (SSR) markers and select distinct and contrasting genotypes for future breeding.
iv) To determine the genetic diversity and relationships among Ethiopia's sesame germplasm collections using seed oil content and fatty acid compositions and diagnostic SSR markers and select genetically unique and promising parental lines for breeding.

Research hypotheses

i) Farmer's perception and their indigenous knowledge on the selection of high-yielding, quantity and quality of seed oil content have direct implications for breeding sesame varieties with better performance and market preference.
ii) Grain yield, oil content and oil yield have high heritabilities and correlations and can be used for effective selection and variety development in sesame.
iii) SSR markers will reveal extensive genotypic variability and complements the phenotypic diversity present among the sesame genotypes.
iv) There is adequate genetic variation for seed oil and fatty acid contents among the sesame genotypes for selection.

Thesis outline

This thesis consists of five chapters, developed according to the specific objectives set above. Chapter 1 is written as a separate review paper, while Chapters 2 to 5 are written as discrete research papers, each following the format of a stand-alone research paper, followed by a general overview of the research and its implications. The literature review and four experimental chapters of the study made the thesis chapters that were condensed into discrete but inter-dependant papers according to the University of KwaZulu-Natal's dominant thesis format. Chapter 2 was published in Sustainability (2021, 13, 11202. https://doi.org/10.3390/su132011202); Chapter 3 in Plant Breeding (2021; 140:331-341. https://doi.org/10.1111/pbr.12911); Chapter 4 in Plants (2021, 10, 1129. https://doi.org/10.3390/plants10061129) and Chapter 5 in the Journal of Food Composition and Analysis (https://doi.org/10.1016/j.jfca.2022.104545).

The outline of the thesis is, therefore, as follows:

1. Thesis introduction
2. Chapter One: Review of Literature
3. Chapter Two: Appraisal of the Sesame Production Opportunities and Constraints, and Farmer-Preferred Varieties and Traits, in Eastern and Southwestern Ethiopia
4. Chapter Three: Genetic Diversity and Association of Yield-Related Traits in Sesame
5. Chapter Four: Genetic Variability and Population Structure of Ethiopian Sesame (*Sesamum indicum* L.) Germplasm Assessed through Phenotypic Traits and Simple Sequence Repeats Markers
6. Chapter Five: Genetic Diversity and Population Structure of Sesame (*Sesamum indicum* L.) genotypes for Seed Oil, and Fatty Acid Compositions

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Chapter 1. Review of Literature

Abstract

Sesame production and productivity are severely constrained by the lack of high-yielding and well-adapted varieties, with non-capsule shattering, resistant/tolerant to biotic and abiotic stresses, lack of modern production and pre-and post-harvest technologies. Unimproved landraces are widely cultivated in sub-Saharan Africa, including Ethiopia. The landrace varieties are low yielders (< 0.6 ton ha^{-1}), but they possess intrinsic seed oil quality characteristics, such as unique aroma and taste. Therefore, current and future sesame genetic improvement programs should integrate yield and quality promoting traits, local adaptation, amenability to machine harvesting, and other industrially essential food and feed attributes for multiple utilities. This can be achieved by integrating the conventional breeding methods, genetic and genomic techniques such as mutation breeding and genomics-assisted breeding, including genome editing. The objective of this review is to document the breeding progress, opportunities, and challenges of sesame with regards to genetic improvement and variety release and deployment with enhanced seed yield and related agronomic traits and oil content and fatty acid compositions. The review highlights sesame's economic values, production status, major production constraints, conventional breeding methods, and genomics-assisted breeding and their integration for accelerated breeding and cultivar development with market-preferred traits.

Keywords: Conventional breeding, genetic variation, genomics-assisted breeding, marker-assisted selection, production constraints, sesame breeding, *Sesamum indicum*

1.1. Introduction

Sesame (*Sesamum indicum* L.; 2n = 2x = 26) belongs to the family Pedaliaceae. It is a predominantly self-pollinating crop with <1% outcrossing (Ashri 2010). Sesame cultivation dates back some 5,500 years ago in the Harappa valley of India (Bedigian and Harlan 1986). Sesame seed oil and derived products serve the food, feed and cosmetics industry globally. Sesame has higher seed oil content at 60% when compared to soybean (~20%), rapeseed (~40%), sunflower (~45%), and groundnut (45-56%) (De la Vega and Hall 2002; Zheljazkov et al. 2008; Wei et al. 2013; Gulluoglu et al. 2016; Dossa et al. 2018). Sesame oil comprises about 85% unsaturated and 15% saturated fatty acids. The unsaturated fatty acids include linoleic acid (~46%) and oleic acid (~38%), while the saturated fatty acids are palmitic acid (~12%) and stearic acid (~4%) (Were et al. 2006; Anastasi et al. 2017; Dossa et al. 2018). The higher quantity of unsaturated fatty acids present in sesame oil have human health benefits believed to be minimising the risks of cardiovascular diseases, cancer, brain, and liver damages (Yen et al. 1990; Yol et al. 2015). These attributes make sesame seed a healthy 'superfood'.

Sesame is widely traded in local, regional and international markets (Myint et al. 2020). A global total of 2.4 million tons of sesame grain was traded in 2020 with a monetary value of 3.2 trillion USD (FAOSTAT 2020). Likewise, sesame consumption is steadily increasing due to high demands related to its unique nutritional values such as higher contents of vitamins (e.g., A and E), minerals, fibre, desirable fatty acids, carbohydrate (~13.5%), and protein (~24%)] (Myint et al. 2020). Furthermore, population pressure, urbanisation and the changing lifestyle have increased the global demand for sesame products (Myint et al. 2020).

About 70% of the world's sesame seed is processed to produce food oil, while the seedcake left after oil processing is used to prepare livestock meals (Myint et al. 2020). The global annual human consumption of sesame is about 65% and 35% in the form of processed food oil and grain, respectively (Morris 2002). In 2020 world sesame grain production was 7.25 million tons (FOSTAT 2020). Sudan is the largest sesame grain producing country with 1,525,104.00 tons per annum, followed by China (896,630.00 tons), Myanmar (740,000.00 tons), the United Republic of Tanzania (710,000.00 tons), India (658,000.00 tons), Nigeria (490,000.00 tons), Burkina Faso (270,000.00 tons) and Ethiopia (260,258.00 tons) (FAOSTAT 2020).

The actual mean grain yield of sesame in sub-Saharan Africa is < 0.6 ton ha^{-1}, which is far below the attainable yield of the crop, up to 4.00 ton ha^{-1} (FAOSTAT 2020). On the other hand, relatively higher grain yield productivity levels were reported from countries like Lebanon (3.29 tons ha^{-1}), Jordan (2.38

tons ha^{-1}), Israel (2.04 tons ha^{-1}), China (1.62 tons ha^{-1}), Tajikistan (1.59 tons ha^{-1}), and Uzbekistan (1.52 tons ha^{-1}) (FAOSTAT 2020). The low yield level in SSA is attributable to the use of unimproved traditional varieties or landraces, the prevalence of biotic and abiotic stresses, and a lack of modern production technologies such as optimal agronomic managing practices row planters, harvesters, and storage facilities. Also, sesame yields are hindered by the indeterminate growth habit of some varieties and capsule shattering and excessive seed loss pre-and post-harvest (Abdellatef et al. 2008; Uzun and Çagirgan 2009; Myint et al. 2020; Teklu et al. 2021a). Nearly all the global sesame varieties are prone to capsule shattering, and they are not suitable to machine harvesting (Khidir, 1972; Langham and Wiemers 2002; Myint et al. 2020). Langham and Wiemers (2002) reported a pre-harvest yield loss of 50% in some sesame varieties due to capsule shattering. Hence, manual sesame harvesting is the method of choice globally, which significantly increases the production and market costs of the produce (Khidir, 1972; Langham and Wiemers 2002;Myint et al. 2020).

Ethiopia is the centre of genetic diversity of sesame (Bedigian 1981; Seegeler 1983). The Ethiopian Biodiversity Institute (EBI) maintains about 5000 accessions of sesame germplasm collections (Teklu et al. 2021b). The production and productivity of the crop in East Africa, including Ethiopia, is severely constrained by the lack of high-yielding and better-adapted varieties to local conditions with less capsule shattering and better seed retention, tolerant/resistant to biotic and abiotic stresses, lack of modern production and pre-and post-harvest technologies (Were et al. 2006). In the region, sesame production relies on unimproved traditional varieties or landraces, which are highly preferred by growers, consumers and markets for unique aroma and taste. These attributes make the traditional varieties attractive to growers, breeders, and local, regional, and international markets. Hence, landrace varieties are an excellent source of genetic variation for sesame pre-breeding and breeding programs globally.

Current and future sesame genetic improvement programs should integrate yield and quality promoting traits, local adaptation, amenability to machine harvesting, and other industrially essential oil and fatty acid profiles for multiple utilities. This can be achieved by integrating the conventional breeding methods, genetic and genomic techniques such as mutation breeding, genomics-assisted breeding, and genome editing. Therefore, the objective of this review is to document the breeding progress, opportunities, and challenges of sesame with regards to genetic improvement and variety release and deployment. The review highlights sesame's economic values, production status, major production constraints, conventional breeding methods, and genomics-assisted breeding and their integration for accelerated breeding and cultivar development with enhanced seed yield and related

agronomic traits and better oil content and fatty acid compositions. Information presented in the paper serves as a guide for current and future sesame research and development programs.

1.2. Global sesame production

Sesame is widely cultivated in tropical and sub-tropical agro-ecologies of the world. The major production regions are Africa, Asia, Latin America and Europe, with production share of 59.05, 36.47, 4.22, and 0.26%, in that order during 2020 (Table 1.1). The global sesame production had increased due to the increased of production area from 7.72 to 14.24 million ha during the last 21 years and the market values of sesame products (FAOSTAT 2020). The leading sesame producing countries with total production area, a share of production and yield are summarised in Table 1.2. Sudan is the leading sesame producer, followed by China, Myanmar, the United Republic of Tanzania, India, Nigeria, Burkina Faso and Ethiopia, with global shares of 21.02, 12.36, 10.20, 9.78, 9.07, 6.76, 3.72 and 3.59%, respectively in 2020 (FAOSTAT 2020).

There has been steady development in the total global sesame production in the past 21 years (1999 to 2020). For instance, Sudan witnessed the most significant production increments that varied from 0.33 million tons (1999) to 1.53 million tons (2020) followed by Burkina Faso (0.01 to 0.27 million tons) and the United Republic of Tanzania (40,000.00 to 0.71 million tons) (FAOSTAT 2020). During the same period, the total sesame of production in Ethiopia, Nigeria, Myanmar, and India varied from 0.02 to 0.26, 0.07 to 0.49, 0.25 to 0.74, and 0.48 to 0.66 million tons, in that order (FAOSTAT 2020). Reportedly, Afghanistan, Sudan, Egypt, India, Myanmar, Paraguay, the United Republic of Tanzania, Nigeria, Burkina Faso and Ethiopia recorded a rapid increase in both cultivated area and total production between 1999 and 2020 (FAOSTAT 2020). The increased sesame production was mainly attributed to the expansion of farmlands and the market values of sesame products (FAOSTAT 2020). Contrastingly, sesame production declined in the Central African Republic, El Salvador, Iraq, Kenya, Morocco, and South Korea between 1999 and 2020 (FAOSTAT 2020). The declined sesame production was mainly attributed to shifting to other crops, the prevalence of several biotic and abiotic stresses, lack of modern production and pre-and post-harvest technologies expansion of farmlands and the market values of sesame products (FAOSTAT 2020).

In the past 21 years, variable sesame yields per unit production area have been recorded globally (FAOSTAT 2020). For example, the yield records in Lebanon varied from 2.08 ton ha^{-1} (1999) to 3.30 ton ha^{-1} (2020), while Jordan recorded 0.92 ton ha^{-1} (1999) to 2.38 ton ha^{-1} (2020). According to FAOSTAT (2020), sesame yields in Mozambique and Venezuela declined between 1999 and 2020 from 0.64 to 0.46 and 0.61 to 0.38 ton ha^{-1}, in that order due to limitation of access to improved technologies and extension services. The lowest average sesame yields were reported in Côte d'Ivoire, Guinea, Central African Republic and Angola at 0.19, 0.23, 0.24 and 0.26 ton ha^{-1}, respectively, from 1999 to 2020. Lebanon, Jordan, Israel and China have recorded the highest average yields of > 1.0 ton ha^{-1} during the same years (FAOSTAT 2020). In the past 10 years, the total annual global sesame production increased from 5.32 million tons (2011) to 7.30 million tons (2020), while the corresponding grain yield varied from 0.78 to 0.79 ton ha^{-1} (Figure 1) (FAOSTAT 2020). Therefore, the average sesame yield is low and stagnant globally. The increasing trend in total sesame production emanated mainly from the expansion of farmlands than grain yield productivity per unit area. The low yield performance of the crop suggests the need for a concerted effort for global sesame genetic improvement to boost grain yield and oil production to meet the soaring demand for oil and derived products.

Table 1.1. Global sesame production by regions in 2020

Region/continent	Area ('000 ha)	Production ('000 ton)	% of world production	Average yield (ton ha^{-1})
Africa	9,692.17	4,282.99	59.05	0.54
Asia	4,064.40	2,645.23	36.47	1.13
Latin America	462.37	306.37	4.22	0.71
Europe	25.56	18.69	0.26	0.88
Australia	NA	NA	NA	NA
World	**14,244.50**	**7,253.28**	**NA**	**0.79**

Source: (FAOSTAT 2020); NA=data not available.

Table 1.2. The top 10 sesame-producing countries in 2020 with the total area, production, and yield globally.

Country	Area ('000 ha)	Production ('000 ton)	% of world Production	Yield (ton ha^{-1})
Sudan	5,173.52	1,525.10	21.02	0.29
China	554.97	896.63	12.36	1.62
Myanmar	1,500.00	740.00	10.20	0.49
The United Republic of Tanzania	960.00	710.00	9.78	0.74
India	1,520.00	658.00	9.07	0.43
Nigerian	621.41	490.00	6.76	0.79
Burkina Faso	450.00	270.00	3.72	0.60
Ethiopia	369.90	260.26	3.59	0.70
Chad	392.24	202.07	2.79	0.52
South Sudan	608.16	189.72	2.61	0.31
World	**14,244.50**	**7,253.28**	**NA**	**0.79**

Source: (FAOSTAT 2020); NA-=data not available.

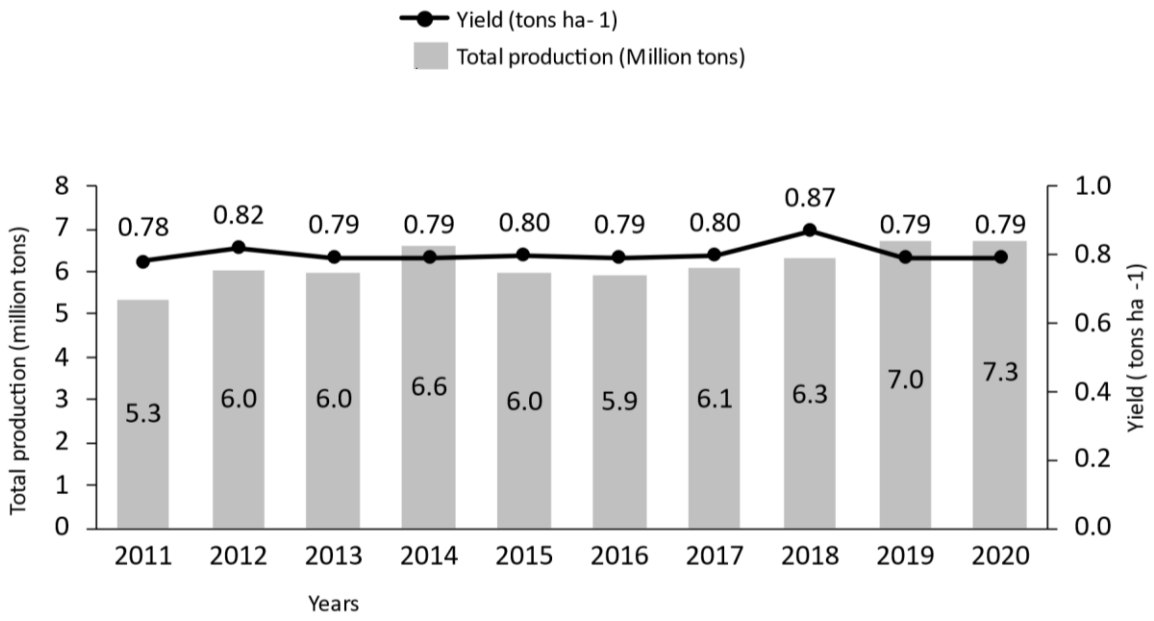

Figure 1. Total sesame production (million tons) and grain yield (ton ha^{-1}) from 2011 to 2020 globally (Adapted from FAOSTAT 2020).

1.3. Constraints to sesame production

The major constraints to sesame production and productivity are lack of high-yielding and well adapted varieties with less capsule shattering and seed loss, even maturity, biotic stresses (insect pests and diseases), tolerant/resistant to abiotic stresses (e.g. drought, waterlogging, salinity, frost and high temperature), modern production technologies and pre-and post-harvest infrastructure (Were et al. 2001; Were et al. 2006; Abdellatef et al. 2008; Nyongesa et al. 2013; Woldesenbet et al. 2015; Anyanga et al. 2017; Dossa et al. 2017a; Tripathy et al. 2019; Yol et al. 2019; Teklu et al. 2021a).

Field insect pests cause a yield loss of 25% in sesame (Weiss 2000). The major insect pests of sesame crop in Ethiopia are webworm (*Antigastra catalaunalis)*, gall midge (*Asphondylia sesame)*, and seed bug (*Elasmolomus sordidus)* (MoA 2018). Seed bug is both field and storage insect pest that cause up to 50% yield loss in storage (MoA 2018). Also, most sesame varieties are attacked by diseases caused by bacteria (e.g. blight caused by *Xanthomonas campestris pv. sesame*), and fungus (e.g. charcoal rot caused by *Macrophomina phaseolina*, stem anthracnose by *Colletotrichum spp.*, mildew by *Erysiphe cichoracearum)*, and viruses (e.g. phyllody*, Orosius albicinctus*) (Myint et al. 2020).

Drought stress is the main yield-limiting constraint in sesame during the vegetative and flowering growth stages (Boureima et al. 2012; Kadkhodaie et al. 2014; Aye et al. 2018; Myint et al. 2020). Yousif et al. (1972) and Tripathy et al. (2019) reported that sesame is sensitive to waterlogging, salinity and low-temperature conditions. Waterlogging leads to reduced plant growth, leaf axils per plant, biomass and net photosynthesis and seed yield (Ucan et al. 2007; Sun et al. 2009).

Cultivation of sesame using varieties with indeterminate growth habits with capsule shattering leads to yield penalty (Were et al. 2006; ; Abdellatef et al. 2008; Nyongesa et al. 2013; Woldesenbet et al. 2015; Anyanga et al. 2017; Dossa et al. 2017a; Myint et al. 2020; Teklu et al. 2021a). Globally, 99% of sesame varieties are susceptible to capsule shattering (Langham and Wiemers 2002; Khidir 1972; Myint et al. 2020). Langham and Wiemers (2002) reported a 50% pre-harvest yield loss owing to capsule shattering. Similarly, Weiss (200) reported up to 50% yield loss at harvesting time due to the nature of capsule shattering of the crop.

Sesame seed loss is common during pre-harvest (e.g. field crop stand) and post-harvest (e.g. harvesting, stacking, drying, threshing, transporting, storage, seed cleaning and packaging) (Gebretsadik et al. 2019). Pre- and post-harvest losses are the confounding factors of reduced yield loss and low market price in sesame production.

Lack of access to post-harvest infrastructure and market and low and variable market prices during harvest are among the critical challenges in sesame value chains (Dossa et al. 2017a; Myint et al. 2020 Teklu et al. 2021a). For instance, in Ethiopia, a 100 kg of sesame grain is traded at 1000–3000 Birr (about 22.3–67 USD) during the harvest period (October to December), while the price increases to 3000–3500 Birr (about 67.0–78 USD) during the off-season (January to September) (Teklu et al. 2021a). There is a need to improve the sesame value chain mainly through incorporating improved and high-yielding varieties into the formal seed system, more extensive use of the best agronomic practices, strengthening the extension services, and developing market infrastructure and on-time market information delivery. These attributes can motivate farmers to produce higher quantities of better-quality seeds to serve the marketplace.

1.4. Sesame breeding

The main goals in sesame breeding programs include high seed yield, seed oil quantity and quality, capsule shattering resistance and high seed retention rate, uniform maturity and tolerance to biotic and abiotic stresses. However, breeding gains in sesame are low and stagnant compared to other oilseed crops such as groundnut and sunflower (Dossa et al. 2017b). Yield is the main driver of sesame breeding, and selection for improved grain yield and yield components remain the key breeding strategies. The main yield-related traits include early (for terminal moisture stress), late (potential condition) and uniform maturity, reduced plant height, higher number of capsules per plant, number of branches per plant, number of seeds per capsule, and heavier thousand-seed weight. Thus far, most sesame breeding programs have largely focused on germplasm characterisation, and varietal evaluation and recommendation using the conventional breeding methods. There is a need to complement phenotyping with other modern breeding strategies such as identifying and discovering new genes, genomic-assisted breeding and gene editing, which are described below.

1.4.1 Progress and achievements in sesame genetic improvement

In the past 20 years, sesame research and development have benefited from conventional breeding methods, including pure line and mass selection, hybridization and mutation breeding. This has led to the development of a number of improved sesame varieties. In the last 40 years, more than 200 improved sesame varieties with high yields, oil quantity and quality, early maturity, and resistance to diseases and insect pests were developed and released globally (Myint et al. 2020).

Genetic and genomic techniques such as genomics-assisted breeding, and genome editing have been markedly used in oil crops research such as in groundnut and rapeseed crops (He et al. 2021). There has been rapid development of genetic tools, particularly molecular markers and their application in genetic diversity studies, marker-assisted breeding, chloroplast genome sequencing, nuclear genome sequencing, RNA sequencing, genome resequencing, haplotype mapping, database development, association mapping, and Genome-Wide Association Studies (GWAS), gene discovery and functional study, genetic mapping and genomics-assisted breeding (Dixit et al. 2005; Wang et al. 2012; Dossa et al. 2017b). Nevertheless, these genomic resources have been widley used in most sesame genetic improvement programs.

A few improved sesame varieties released in different countries for agronomic and other valuable traits are summarised in Table 1.3. India and China have each developed more than 50 improved cultivars over the last 40 years (Hodgkin et al. 1999). A total of 32 improved sesame varieties were developed and released by the Ethiopian Institute of Agricultural Research (EIAR) through mass selection from among the local germplasm collections, introduction and crossing of selected parents since 1976 (MoA 2019). Among the EIAR's released varieties Humera-1 and Setit-1 are widely grown by farmers for their early maturity, better yield performance (about 1 ton/ha), and broad adaptability (Teklu et al. 2021a). However, the yield performance of these varieties is below the reportedly attainable yields of the crop. Some 29 sesame varieties were released in Myanmar in the past 42 years. These varieties were bred for early maturity, white seed color, high yield, and seed oil content (Myint et al. 2020). In Myanmar the following varieties were released: Ju-Ni-Poke, Me-Daw-Let-The, Gwa-Taya and Gwa-KyawNet. The varieties were reportedly stable yielding. Ju-Ni-Poke, Shark-Kale, Hnan-Ni 25/160, Yoe-Sein, Boat-Hmway, Kye-Ma-Shoung, Selin-Boat-Taung, Magway-Ni 50/2, and Nyaung-Aing had relatively higher seed oil content (≥ 55%) (Myint et al. 2020). In Bulgaria, four sesame varieties, namely Victoria, Aida, Valya, and Nevena were successfully developed for amenable to mechanised harvesting and with mean grain yield of 1.35 tons ha^{-1} through a research collaboration between plant breeders and agricultural engineers over the last 30 years (Stamatov et al. 2018). In Kenya, sesame cultivars such as SIK 031 and SIK 013 showed resistance to the white leaf spot disease, whereas SIK 031 and SPS 045 showed resistance to angular leaf spot disease (Nyanapah et al. 1995). The two varieties were released by the department of crop science, the University of Nairobi (Ayiecho and Nyabundi 1994).

Table 1.3. Some improved sesame varieties released globally for desirable agronomic and seed oil traits

Variety	Pedigree	Trait	Country	Year of release	References
Sin-Yadana 4	-	Good export quality	China	1994	Myint et al. 2020
Ju-Ni-Poke	-	Stable yield & high oil content	Myanmar	1994	Myint et al. 2020
Me-Daw-Let-The	-	Stable yield & high oil content		1994	
Gwa-Taya	-	Stable yield		1994	
Gwa-Kyaw-Net	-	Stable yield		1994	
Humera-1	ACC.038 sel.1	Early maturity, high yield and broad adaptability	Ethiopia	2010	Teklu et al. 2021a
Setit-1	col sel p#1	Early maturity,high yield and oil content and broad adaptability		2010	
Dangur	E.W.013.(8)	High oil content	Ethiopia	2015	MoA 2019
BaHaNecho	W-109/WSS/ (Acc-EW-012(5)	High yield and oil content		2016	
BaHaZeyit	W- 119/WSM/ (Acc-EW-023(1)	High yield and oil content		2016	
Waliin	BG-004-1	High yield and oil content		2016	
RAMA	'Khosla' local	Medium seed size and brown seed color	India	1989	Pandey et al. 2015
OSC-593	-	White seed color		1995	
TKG-352	-	White seed color		1995	

MoA= Minstry of Agriculture; -=data not available

1.4.2 Sesame genetic resources

Sesame genetic resources are the key sources of genetic variation that would lead to the effective selection of desirable traits for current and future genetic improvement programs. Genetically diverse sesame germplasm resources are collected and maintained by different local and international gene banks in sesame improvement programmes (Table 1.4).

1.4.3 Sesame gene banks

A significant number of sesame genetic materials involving cultivated and wild species are maintained in different gene banks globally (Table 1.4) (Zhang et al. 2012). About 95% of the sesame genetic resources are maintained in Asia, while 5% in the United States of America (Table 1.4). The major sesame gene banks are the National Bureau of Plant Genetic Resources (NBPGR)/India, the National Agrobiodiversity Center, Rural Development Administration/South Korea (Park et al. 2015), the Oil Crops Research Institute, Chinese Academy of Agricultural Sciences/China and the United States of

America – US Department of Agriculture - Agricultural Research Service - Plant Genetic Resource Unit (USDA -ARS-PGRU) (Wei et al. 2015).

A total of 27,283 sesame genetic materials are preserved in the gene banks in India, South Korea, China and the United States of America (Table 1.4). Several African countries, such as Ethiopia, Nigeria and Sudan, have small-scale gene banks (Dossa et al. 2017b; Teklu et al. 2021b). The African sesame gene banks have reservoirs of a reasonable amount of genetic resources, but vital core collections (CC) are yet required in the region for efficient exploration and utilisation of novel genetic variation (Hodgkin et al. 1995). Currently, there are three CC of sesame globally of which 362 accessions in India (Bisht et al. 1998), 453 in China (Zhang et al. 2000) and 278 in South Korea (Park et al. 2015). The collected accessions are sources of valuable genetic variation for genetic improvement and analysis of useful agronomic, seed oil and fatty acid traits. The Asian sesame genetic resources have been relatively well charachterised and preserved compared to African germplasm (Dossa et al. 2016a). Therefore, there is a need to collect and characterise the cultivated and wild forms of the sesame species from Africa. This will lead to an establishment of CC for efficient conservation and exploitation of the novel genetic variation in Africa and internationally.

Table 1.4. The major sesame gene banks globally.

Country	Institution	Total number of accessions	Website	Reference
India	National Bureau of Plant Genetic Resources	10,359	www.nbpgr.ernet.in	NBPGR 2021
China	Oil Crops Research Institute	> 8000	http://www.sesame-bioinfo.org/phenotype/index.html	OCRI 2021
South Korea	National Agrobiodiversity Center, Rural Development Administration	7,698	http://www.rda.go.kr/foreign/ten/	NACRDA 2021
United States of America	USDA-ARS-PGRU	1,226	www.ars.usda.gov	USDA-ARS-PGRU 2021

USDA-ARS- PGRU= United States Department of Agriculture - Agricultural Research Service - Plant Genetic Resource Unit C

1.4.4 Landraces and improved sesame varieties

Landraces are a valuable source of genetic diversity and possess important traits for pre-breeding and breeding programs (Lopes et al. 2015). Landraces are widely cultivated in developing countries, often using traditional farming systems in various harsh growing environments and diseases and insect pest

pressures (Picha et al. 2017). Landraces are useful to integrate unique traits into elite lines and pipeline breeding programmes. This will enhance sustainable sesame production to meet the standard quality requirements of the local and international markets as well as for environmental adaptation and mitigation against climate change.

Despite its economic value in the food and oil industries and export markets, sesame remains largely under-researched and underutilised in Africa, including Ethiopia (Teklu et al. 2021b). For instance, in Ethiopia the current sesame production relies on a limited number of genetically unimproved landrace varieties selected by farmers. The landrace variety "Hirhir" is widely cultivated by smallholder and medium-to-large commercial farmers in the country. The variety has a low yield level but possesses novel seed oil quality characteristics, such as aroma and taste (Teklu et al. 2021b).

Sehr et al. (2016) characterised Ugandan sesame landraces and reported a narrow genetic variation when assayed with morphological and molecular data. Promising sesame landraces were selected amongst Myanmar collections that possessed useful traits such as high seed yield, oil quality and quantity (Myint et al. 2020). In China, novel genome sequence data have been generated for two sesame landraces (Baizhima and Mishuozhima) and three improved varities (Zhongzhi 13, Yuzhi 11, and Swetha). The genome sequence of these genetic resources is a useful reference for sesame breeders, geneticists and biologists (Wei et al. 2016). The two landraces are originally cultivated in Hainan and Zhejiang provinces in China (Wei et al. 2015). The sequence information revealed that improved varieties contain genes mainly related to yield and quality, while the landraces contain genes involved in environmental adaptation (Wei et al. 2016). The sesame landrace genetic resources present in the centres of origin or diversity need to be systematically collected and evaluated based on seed yield and yield-related traits, oil quantity and quality for breeding and conservation.

1.5. Breeding methods and associated technologies for sesame improvement

1.5.1 Conventional breeding

Sesame improvement and variety development has been dependent on conventional breeding methods (Ashri 1987). In the past, limited genomic tools were used due to limited access to the technology and lack of consolidated genetic database on important agronomic traits and genes conditioning key traits (Dossa et al. 2017b).

Conventional sesame breeding has been the source of creating new genetic variations (Furat and Uzan 2010; Akbar et al. 2011; Gidey et al. 2012; Teklu et al. 2014; Hika et al. 2014, 2015; Abdou et al. 2015, Harfi et al. 2018; Teklu et al. 2021c). Previous reports indicated the presence of genetic variation for important traits such as reduced days-to-50% flowering, days-to-75% maturity, capsule filling period, short plant height, greater internode length, a higher number of primary branches per plant, number of secondary branches per plant, number of capsules per plant, number of seeds per capsule, increased capsule length, capsule width, stem height to 1st branch, distance from lowest branch to 1st capsule, thousand-seed weight, biomass yield per hectare, harvesting index, and grain yield per hectare in sesame. These traits are useful for sesame variety descriptions, agro-morphological and genetic analyses and breeding programmes (Furat and Uzan 2010; Akbar et al. 2011; Gidey et al. 2012; Teklu et al. 2014; Hika et al. 2014, 2015; Abdou et al. 2015, Harfi et al. 2018; Teklu et al. 2021c).

Understanding the prevailing genetic variability, magnitude of heritability, and the correlation of agronomic traits play a vital role in the effective use of germplasm (Myint et al. 2020). Heritability estimates measure the extent of genetic variation and advancement through phenotypic selection (Johnson et al. 1955). High heritability with high genetic advance are preconditions for effective phenotypic selection in conventional breeding (Panse 1957). Divya et al. (2018) in India and Aye and Htwe (2019) in Myanmar reported high heritability and genetic advance for plant height and number of capsules per plant in sesame.

The magnitude of association amongst economic phenotypic traits guides the selection efficiency in sesame breeding. Highly correlated traits ensure higher selection response and yield gains (Abraha et al. 2016). Sesame grain yield exhibited a highly significant positive correlation with plant height, number of primary and secondary branches, number of capsules per plant and thousand-seed weight (Akbar et al. 2011; Hika et al. 2014; Harfi et al. 2018; Aye and Htwe 2019; Teklu et al. 2021b). Analysing trait correlations in sesame breeding populations is vital for effective selection for grain yield and yield components.

1.5.2 Mutation breeding

Mutation breeding is helpful in enhancing genetic variation to complement conventional breeding programs (Maluszynski et al. 1995; Muduli and Mishra 2007). It is a different and innovative method for producing distinctive phenotypes that can be used in breeding. The method requires low cost and play a critical role in crop improvement program. Most of the mutations are lethal due to large

deletion, inversion, duplication and changes in chromosome number (Ashri 1997). Induced mutagenesis has made significant contributions to sesame breeding. Some 147 sesame mutants with desirable economical traits were registered through different national sesame improvement programmes globally (Ashri 1997; MVD 2022).

Table 1.5 lists some of the reportedly improved sesame varieties with economic traits derived through mutation induction globally. For example, Senai white 48 and Cairo white 8 mutant varieties were developed and released in Egypt (Ashri 1997). These varieties are grown by the farmers for their white seed color and non-branching habits, respectively. In India, the variety Usha was developed through chemical mutagenesis and released for its increased yield. Lee and Choi (1985) reported sesame mutant varieties with high oil content and disease resistance in South Korea. Kang (1997) reported higher oleic content and phytophthora blight tolerance in a mutant variety Seodun in South Korea. In Seri Lanka, the mutant variety ANK-2 was developed and released, possessing adequate diseases resistance (Pathirana 1992). Capsule shattering is amongest the yield reducing factors in sesame (Teklu et al. 2021a). Hence, future mutation breeding programs should target this grand challenge.

Table 1.5. Some sesame varieties developed through induced mutation with traits descriptions

Variety name	**Trait**	**Country**	**Year of release**	**Reference**
NIAB-Pearl	High capsules per plant	Pakistan	2017	MVD 2022
NIAB-Sesame 2016	High oil content		2016	
Binatil-3	High yield	Bangladesh	2013	MVD 2022
Cairo white 8	Non-branching	Egypt	1992	Ashri 1997
Senai white 48	Seed colour		1992	
Kalika	Short stature	India	1980	
UMA	Uniform maturity		1990	Ashri 1997
USHA	High yield		1990	
Babil	Earliness	Iraq	1992	
Rafiden	Earliness		1992	Ashri 1997
Eshtar	Capsule size		1992	
Ahnsan	Disease resistance	South Korea	1985	
Suweon	Lodging and disease resistance		1991	Lee and Choi
Yangbaek	High oil content		1995	(1985
Pungsan	Determinate growth habit and high seed retention		1996	Ashri 1997
Seodun	High oleic acid content and phytophthora blight tolerance		1997	
ANK-2	Disease resistance	Sri Lanka	1995	Pathirana 1992

MVD= Mutant Variety Database.

1.5.3 Genomics-assisted breeding

Genomic tools and techniques are key for trait discovery and molecular breeding (Khan et al. 2020). Various databases for sesame genomics are summarised in Table 1.6. A study by Wei et al. (2016) reported a genome size of 554.05 Mbp in sesame, of which the core and dispensable genomes were 258.79 and 295.26 Mbp, respectively. The sesame genome consists of 26,472 orthologous gene clusters, of which 15,890 genes were variety-specific (Myint et al. 2020). The sesame pangenome, the entire set of genes, is a vital genomic resource for sesame improvement programs and genetic analysis.

Table 1.6. Online genomic resources for sesame.

Database	Website	Utility	Reference
Sinbase	http://www.ocri-genomics.org/Sinbase/index.html.	Genomics/Comparative genomics/Genetics/Phenotypes etc	Wang et al. 2014
SesameHapMap	http://202.127.18.228/SesameHapMap/	Genome wide SNP	Wei et al. 2015
SesameFG	http://www.ncgr.ac.cn/SesameFG	Genomics/Evolution/breeding/comparative genomics/Molecular markers/Phenotypes/Transcriptomics	Wei et al. 2017
SisatBase	http://www.sesame-bioinfo.org/SisatBase/	Genome wide SSR	-
The Sesame Genome Project	http://www.sesamegenome.org	Genomics	Zhang et al. 2013b
Sesame Germplasm Resource Information Database	http://www.sesame-bioinfo.org/phenotype/index.html	Plant phenotype	-
NCBI∗	http://www.ncbi.nlm.nih.gov/genome/?term=sesame	Versatile	-
ocsESTdb∗	http://www.ocri-genomics.org/ocsESTdb/index.html	Seed expression sequence tags/comparative genomics	Ke et al. 2015
PTGBase∗	http://www.ocri-genomics.org/PTGBase/index.html	Tandem duplication/evolution	Yu et al. 2015
PMDBase∗	http://www.sesame-bioinfo.org/PMDBase	SSR information	Yu et al. 2016

∗These databases involved several species including sesame

1.5.3.1 Genetic diversity analysis

Molecular markers are highly reliable genetic tools that complement phenotypic selection for breeding (Jones et al. 2009). Knowledge on the genetic diversity and population structure of germplasm collections is vital for genetic analysis, breeding and conservation (Thomson et al. 2007). Genetic diversity in sesame has been explored using several DNA markers. Various studies assessed the genetic diversity of sesame accessions globally using amplified fragment length polymorphism (AFLP) (Laurentin et al. 2006; Laurentin et al. 2007), random amplified polymorphic DNA (RAPD) (Bhat et al. 1999; Ercan et al. 2004; Abdellatef et al. 2008), inter simple sequence repeat (ISSR) (Kim et al. 2002; Nyongesa et al. 2013; Woldesenbet et al. 2015), microsatellites or simple sequence repeat (SSR) (Gebremichael et al. 2011; Zhang et al. 2012; Wei et al. 2014; Dossa et al. 2016a; Wei et al. 2016; Asekova et al. 2018; Araújo et al. 2019; Teklu et al. 2021b), and single nucleotide polymorphisms

(SNPs) (Basak et al. 2019; Tesfaye et al. 2022). The SSR markers are widely used in sesame genetic analysis and breeding for their ability to detect higher degrees of polymorphism, higher reproducibility, co-dominance and abundant coverage of the genome. However, they are expensive and time-consuming, especially when the creation of a genetic library is needed (Wei et al. 2016).

Table 1.7 lists some of the polymorphic SSR markers developed for sesame breeding. SSR markers play an important role in genetic diversity research, population genetics, linkage mapping, comparative genomics, and association analysis (Dixit et al. 2005; Wei et al. 2011; Pandey et al. 2015; Dossa et al. 2016a; Asekova et al. 2018). Some SSR primers such as ZM_20, ZM_21 and ZM_22, followed by ZM_11, and ZM_45, were more polymorphic (with polymorphic information content [PIC] of ≥0.80).

There are several sesame genetic diversity studies conducted using morphological and molecular markers. Frary et al. (2015) conducted genetic diversity study using morphological traits and RAPD markers among 137 Turkish sesame germplasms which led to the development of a core collection. One hundred twenty-one Ugandan sesame landraces were investigated using 24 SSRs markers and the results showed incongruence between morphological and molecular data (Sehr et al., 2016). Anyanga et al. (2017) reported a medium genetic differentiation among 85 test germplasm sourced from different countries at the National Semi-Arid Resources Research Institute (NaSARRI) in eastern Uganda. Also, Pandey et al. (2015) analysed a worldwide germplasm collection predominantly Indian accessions and reported a high genetic diversity within the germplasm. But there was non-significant correlation between phenotypic and molecular marker information. Pham et al. (2011) reported a substantial amount of genetic diversity present in 12 Vietnamese and Cambodian populations. Twenty-seven Iranian sesame accessions were characterised that revealed large genetic variability (Tabatabaei et al. 2011). Teklu et al. (2021b) reported a wide genetic diversity among 100 Ethiopian sesame genotypes when assessed using 27 SSR markers.

Limited genetic studies have been conducted on wild related species of the genus *Sesamum* (Dossa et al. 2017b). Nyongesa et al. (2013) reported a high genetic diversity within wild sesame species using six ISSR markers. Uncu et al. (2015) discovered a high rate of SSR marker transferability between *S. indicum* and *S. malabaricum*, supporting the designation of the two taxa as cultivated and wild forms of the same species. The wild species of sesame possess genes related to resistance to biotic and abiotic stresses and broad adaptability (Joshi, 1961). In sesame, the introgression of valuable genes from wild related species into cultivars through conventional breeding has not been so far successful

due to the post-fertilization barrier (Tiwari et al. 2011). This can be achieved by integrating the conventional and mutation breeding methods and genomic techniques such as molecular breeding, genomic-assisted breeding, and genome editing.

Table 1.7. Some polymorphic SSR markers developed for genetic analysis in sesame.

	Marker sequence		
Primers	**Forward primer sequence**	**Reverse primer sequence**	**References**
GBssr-sa-05	TCATATATAAAAGGAGCCCAAC	GTCATCGCTTCTCTCTTCTTC	Dixit et al. 2005
GBssr-sa-08	GGAGAAATTTTCAGAGAGAAAAA	ATTGCTCTGCCTACAAATAAAA	
Sesame-09	CCCAACTCTTCGTCTATCTC	TAGAGGTAATTGTGGGGGA	
GBssr-sa-33	TTTTCCTGAATGGCATAGTT	GCCCAATTTGTCTATCTCCT	
GBssr-sa-123	GCAAACACATGCATCCCT	GCCCTGATGATAAAGCCA	
GBssr-sa-182	CCATTGAAAACTGCACACAA	TCCACACACAGAGAGCCC	
GBssr-sa-184	TCTTGCAATGGGGATCAG	CGAACTATAGATAATCACTTGGAA	
SSR-ES-12	GCTGAGGAGTCTTGAAGCAGA	CAAAATCCCCCAACTCGATA	Pandey et al. 2015
SSR-ES-15	TGCAGGAATGAACTCAAGGA	ACCTTATTCCCAGCCCACTT	
ZM_2	CTTCTTGAAGTTCTGGTGTTG	ATTCTTGGAGAAAGAGTGAGG	Wei et al. 2011
ZM_3	ATCACCACACACTGACACAG	CGTGTCTGAGAATCCAATATC	
ZM_6	GGTGTGTTCTCTCTCTCACAC	GGGCTGCTCAATAAATGTAG	
ZM_7	ATCCTCTGCTCCTAACTTCAT	TCTGGTACTATCCTCAAGCAA	
ZM_10	ATGCCCATCTCCATATACTCT	AATTCTTGCCTGACTCTACG	
ZM_11	GGATTCTCTAGACATGGCTTT	AACGCAGAATTCTCTCCTACT	
ZM_12	ATTGCTGTGCAATCCTTATC	ATCTCTTTCTACCACCACGTT	
ZM_13	GCAGAAGGCAATAAAGTCAT G	GCGTCAGAAGAAAAATACTG	
ZM_14	GGAAGGCGAGTTGATAGATAA	CATGGGATGTTCAAAGAACT	
ZM_17	CTTGCTTCCTCTTTTCTCTCT	ACACTGTACTCAGCGGATTT	
ZM_18	AATACCCTTCAGTATTCAGGTG	CAACAACACAAACACTGCTAC	
ZM_20	GGGATGTTGATAGAGATGTTG	TCTTTCACTCTCACACACACA	
ZM_21	CTCTCTCTCTCTGCTGTTTCA	GCCATACGATCTCAAAATCAC	
ZM_22	ACCACCGATCTACTCACTTTT	CCACTGCACACTACAGTTTTT	
ZM_30	CACTCCACTCATTATCCAAAG	CAAGACACAACTGACACGTAA	
ZM_34	AAGTCCCTTTTCAAGCAATC	GAGAGAGGAAAATGCAGAGAG	
ZM_39	AGAGGCAGAGGAGTTGATAAT	CTTAACTGTAACTCCCTTTTCG	
ZM_40	CGAAAAGGGAGTTACAGTTAAG	CTTCCTCTCCTATCATCCTGT	
ZM_44	GTCTTAAGCCCTCTTAGTTCC	GAAAACCTTCAATGTCAGGA	
ZM_45	GCAAAATCTCTGTTGTCTCAG	GTGTTCCTACCACTCAACACA	
ZM_47	GTTTCCAGGTCTATTCCTTTG	AGGTAGAGCTAATCCTTACCG	

1.5.3.2 Quantitative trait loci (QTL) analysis

Quantitative trait locus (QTL)-analysis detects major genetic regions of a target quantitative trait in a population (Zhang et al. 2020). Table 1.8 summarises some quantitative trait loci (QTLs) of target traits identified for sesame breeding. QTL maps are useful for discovering, dissecting, and manipulating the genes responsible for simple and complex traits in crop plants (Tanksley et al. 1992). A high-quality genetic map improves genome assembly and provides a foundation for gene mapping that underlie agronomic traits of important oil crops such as sesame (Wang et al. 2016).

Table 1.8. Quantitative trait loci (QTL) and associated phenotypic traits in sesame.

Traits	Name of QTL	Markers type	Markers number	Mapping population	Reference
		Production enhancement			
Grain yield	Qgn-1, Qgn-6,	SLAF	9378	150 BC1	Mei et al. 2017 Zhang et al. 2013b Wang et al. 2016
Number of seeds per capsule	Qgn-12				
Thousand-seed weight	Qtgw-11				
Seed coat color	QTL-1, QTL11-1, QTL11-2, QTL13-1				
Seed coat color	qSCa-8.2, qSCb-4.1, qSCb-8.1, qSCb-11.1, qSCl-4.1, qSCl-8.1, qSCl-11.1, qSCa-4.1 and qSCa-8.1	SLAF SNP	1233	107 F2 430 Recombinant inbred lines (RILS, F8)	-
Seed coat color	SiPPO (SIN_1016759)	SSR	400	500 RILs (F6)	Wei et al. 2016
Plant height	Qph-6 and Qph-12	SNP	1,800,000	705 worldwide accessions	Wei et al. 2015
Semi-dwarf plant phenotype	QTL (qPH-3.3), Gene [SiGA20ox1(SIN_1002659)]	SNP SSR	400	430 RILS (F8) 500 RILs (F6)	Wang et al. 2016 Wei et al. 2016
Plant height	SiDFL1 (SIN_1014512) andSiILR1 (SIN_1018135)	SNP	1,800,000	705 worldwide accessions	Wei et al. 2015
Number of capsules per plant	Qcn-11	SNP SSR InDels	1190 22 18	224 (RIL), F8:9	Wu et al. 2014
First capsule height	Qfch-4, Qfch-11, and Qfch-12				
Capsule axis length	Qcal-5 and Qcal-9				
Capsule length	Qcl-3, Qcl-4, Qcl-7, Qcl-8, and Qcl-12				
Number of capsules per axil	SiACS (SIN_1006338)	SNP	1,800,000	705 worldwide accessions	Wei et al. 2015
Mono flower vs. triple flower	SiFA	SLAF (Marker58311, Marker34507, Marker36337)	9378	150 BC1	Mei et al. 2017
Flowering time	SiDOG1 (SIN_1022538) and SiIAA14	SNP	-	705 sesame accessions	Wei et al. 2015
Determinate growth habit	gene SiDt (DS899s00170.023)	NP	30,193	120 F2	Zhang et al. 2016
Branching habit	SiBH	SLAF (Marker129539, Marker41538, Marker31462)	9378	150 BC1	Mei et al. 2017
Recessive GMS	Recessive GMS geneSiMs1	AFLP markers P01MC08, P06MG04, P12EA14	-	237 NILs (Near-Isogenic Lines)	Zhao et al. 2013
Dominant GMS	SBM298 and GB50	SSR	1500	Noval GMS line W1098A (Backcrossing and sib-mating; BC2F6	Li et al. 2014

Table 1.8. Continued

Traits	Name of QTLs	Markers type	Markers number	Mapping population	Reference
		Stress related			
Waterlogging tolerance	qEZ09ZCL13, qWH09CHL15, qEZ10ZCL07, qWH10ZCL09, qEZ10CHL07, and qWH10CHL09	SSR (ZM428) closely linked toqWH10CHL09	113	206 RIL F6	Yan et al. 2014 Wang et al. 2016
Drought tolerance	TFs (Transcription Factors) families (AP2/ERF and HSF)	-	-	-	Komivi et al. 2016 Dossa et al. 2016b
Tolerance to drought, salinity, oxidative stresses and charcoal rot	Osmotin-like gene (SindOLP)	-	-	-	Chowdhury et al. 2017
		Genes for oil traits			
Sesamin production	SiDIR (SIN_1015471), SiPSS (SIN_1025734)	SNP	1,800,000	705 worldwide accessions	Wei et al. 2015
Oil content	SIN_1003248, SIN_1013005, SIN_1019167, SIN_1009923 SiPPO (SIN_1016759) SiNST1 (SIN_1005755)				
Fatty acid composition	SiKASI (SIN_1001803), SiKASII (SIN_1024652), SiACNA (SIN_1005440), SiDGAT2 (SIN_1019256), SiFATA (SIN_1024296), SiFATB (SIN_1022133), SiSAD (SIN_1008977), SiFAD2 (SIN_1009785)				
Sesamin and sesamolin content	SiNST1 (SIN_1005755)				
Protein content	SiPPO (SIN_1016759)				

SLAF: specific length amplified fragment sequencing; SNP: single nucleotide polymorphism; SSR: simple sequence repeat; AFLP: amplified fragment length polymorphism; Indels: insertion–deletions; GMS: genetic male sterility

1.5.3.3 Next-generation sequencing

Next-generation sequencing (NGS) has revolutionised genomic and transcriptome research. Sequencing tools are valuable for the discovery, validation and assessment of genetic markers in diverse populations (Davey et al. 2011). Quantitative trait loci (QTL) NGS technologies have significantly enhanced the efficiency and costs of genotyping in several model crop plants (Davey et al. 2011).

NGS allowed for the rapid construction of high-density or ultra-dense single nucleotide polymorphism (SNP) genetic maps for gene identification (Ganal et al. 2011; Sim et al. 2012; Wang et al. 2015; Zhao et al. 2015). Genetic research on sesame has steadily progressed in the last few years with the development of the NGS technology. Six high-density molecular genetic maps have been constructed and are currently being used for sesame genome assembly and map-based gene cloning (Zhang et al. 2013a; Zhang et al. 2013b, Wu et al. 2014; Miao and Zhang et al. 2016; Wang et al. 2016; Zhang et al. 2016; Mei et al. 2017). Ultra-dense SNP genetic map using the whole genome re-sequencing is believed to enhance gene cloning and genomics research in sesame (Zhang et al. 2016; Zhang et al. 2018). Two sesame genes, Sidt1 controlling inflorescence determinacy and Sicl1 controlling leaf curling and capsule indehiscence were successfully cloned using the linkage mapping method and candidate variants screening (Zhang et al. 2016; Zhang et al. 2018). The NGS platform in sesame breeding programmes will assist in the rapid development of genomic tools for genetic improvement and cultivar development, and commercialisation.

1.5.3.4 Genetic engineering and genome editing

Genetic engineering techniques involve various innovative approaches that can complement conventional breeding in sesame (Myint et al 2020). Genetic transformation of traits would be an ideal opportunity to transfer some functional genes into sesame's elite cultivars, including for capsule shattering resistance. Some successful efforts have been made on sesame genetic transformation, including target gene insertion and new variety development (Yadav et al. 2010; Al-Shafeay et al. 2011; Chowdhury et al. 2014)). The authors reported up to 42.66% transformation efficiency using the *Agrobacterium*-mediated transformation technique. Improved transformation efficiency will enhance sesame genetic engineering for precision and speeding breeding. Studies on the transfer of candidate

genes conditioning oil quality traits and abiotic stress tolerance into elite sesame cultivars are in progress at the Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences (OCRI-CAAS), China (Dossa et al. 2017b). The first study on the functional analysis in transgenic sesame for tolerance to drought, salinity, oxidative stresses, and the charcoal rot pathogen was reported by Chowdhury et al. (2017).

Genome editing, also known as targeted gene modification, is a technique for generating new allelic variants in the genomes, including crop plants (David and Repkova 2017). The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-based genome editing systems, such as CRISPR/Cas9, CRISPR/Cpf1, base editing system, and prime editing system, have brought promises for genetic improvement programs of crop plants, including sesame (Zhu et al. 2020). CRISPR-based genome editing technology can alter single or multiple target genes, including in polyploid oil crops such as canola/rapeseed [*Brassica napus* L., 2n = 4× = 38, AACC]. CRISPR-based genome editing has led to the development of stably inherited knock-out mutants of canola (Zhang et al. 2019). The CRISPR technology has shown promise in oil crops genetic improvement, including canola/rapeseed and groundnut crops (He et al. 2021). Nevertheless, there are limited reports on CRISPR-based genome editing technology in sesame and sunflower owing to their unique genomes and recalcitrance to genetic transformation (He et al. 2021). This suggests that CRISPR-based genome editing technology has shown its incomparable superiority in genetic improvement and breeding of oil crop. Currently there is public and regulatory questions regarding the risk of genetically modified (GM) crops attributable to a lack of evidence for their adverse effects on human health or the environment (Dale et al. 2002; Snell et al. 2012; Nicolia et al. 2014). Globally, researchers are investigating new breeding technologies that may avoid the public controversy that hinders the further widespread use of GM crops (Lusser et al. 2011). Therefore, there is a need for vibrant public advocacy and regulatory mechanisms on the beneficial effects of genetically modified crops.

1.6. Market-driven breeding in sesame

The success of a crop breeding program is measured, among other factors, by the adoption rate of the new varieties by farmers and their markets. Farmers are the main actors in agriculture enterprises, with a wealth of indigenous knowledge about their crops, farming systems, and production constraints and they have their own coping mechanism and means to adopt a technology (Altieri and Koohafkan 2018). Plant breeders are required to incorporate the knowledge and opinions of farmers in the

planning and management of their breeding programmes (Chambers 1992). Several socio-economic studies were conducted on sesame to document the production opportunities and constraints, as well as farmer- and market-preferred varieties and traits, as a guide for breeding and large-scale production. Dossa et al. (2017a) in Senegal and Mali examined the socio-economic aspects of sesame to guide production, research and policies. The authors identified lack of market linkage, a decline in soil fertility, limited access to land, drought stress, backward agricultural implements, lack of extension service, and limited access to agricultural inputs as the essential constraints for sesame production in both countries. In Myanmar, the use of low-yielding varieties, insect pests, post-harvest loss, drought, and salinity stresses were regarded as the overriding sesame production constraints (Myint et al. 2020). A recent participatory rural appraisal study conducted in Ethiopia identified that lack of access to improved seeds, low yield, diseases, low market price, insect pests, lack of market information, and high cost of improved seed as the most important production constraints to sesame (Teklu et al. 2021a) White seed color, increased seed size, true-to-type seed, high oil content, and increased thousand-seed weight are identified as the most critical sesame market-preferred traits in Ethiopia (Teklu et al. 2021a).

1.7. Conclusion and outlook

Breeding gains for sesame seed and oil yields and fatty acid composition is relatively low due to the limited research and development support compared with other traditional oilseed crops. A limited number of improved sesame varieties with high yields, oil quantity and quality, early maturity, and resistance to diseases and insect pests were developed and released globally. Sesame improvement has primarily focused on conventional breeding through germplasm characterisation, selection and variety recommendation. There is need to develop new, climate-smart, capsule shattering resistant sesame varieties that meet the quality requirements of the local and international markets. Therefore, current and future sesame genetic improvement programmes should integrate yield and quality promoting traits, local adaptation, machine harvesting, and other industrially essential attributes for multiple utilities. This can be achieved by integrating the conventional and mutation breeding methods and genomic techniques such as molecular breeding, genomic-assisted breeding, and genome editing. Additionally, there is a need for vibrant public and private sector sesame breeding programs and seed industry. Genetic and advanced genomic resources, increased investment for research and development by public and private sectors will enhance the dissemination and adoption of improved technologies for sustainable production and economic gains from sesame enterprises.

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Chapter 2. Appraisal of the sesame production opportunities and constraints, and farmer-preferred varieties and traits, in Eastern and Southwestern Ethiopia

Abstract

Sesame (*Sesamum indicum* L.) is an important oilseed crop with well-developed value chains. It is Ethiopia's most valuable export commodity after coffee (*Coffea arabica* L.), contributing to socioeconomic development. The productivity of the crop is low and stagnant in Ethiopia and other major sesame growing regions in sub-Saharan Africa (<0.6 t/ha) due to a multitude of production constraints. The objective of this study was to document sesame production opportunities and constraints, as well as farmer- and market-preferred varieties and traits, in eastern and southwestern Ethiopia as a guide for breeding and large-scale production. A participatory rural appraisal (PRA) study was conducted in two selected sesame growing regions (Oromia and Southern Nations, Nationalities, and Peoples' (SNNP) Region) and four districts in Ethiopia. Datawere collected from 160 and 46 sesame farmers through semi structured questionnaires and focus group discussions. Sesame is grown by all respondent farmers in the study areas for food and as a source of cash. Most respondent farmers (56%) reported cultivating sesame using seeds of unknown varieties often sourced from the informal seed sector. About 83% of the respondents reported lack of access to improved seeds as the most important production constraint, followed by low yieldgains from cultivating the existing varieties (reported by 73.8% of respondents), diseases (69.4%), and low market price (68.8%). Other production constraints included insect pests (59.4%), lack of market information (55%), and high cost of seed (50%). The above constraints were attributed to the absence of a dedicated breeding programme, lack of a formal seed sector, poor extension services, and underdeveloped pre- and post-harvest infrastructures. The most important market-preferred traits of sesame included true-to-type seed (reported by 36.3% of respondents), white seed colour (28.8%), andhigh seed oil content (23.8%). The vital farmer-preferred attributes included reasonable market price (reported by 11.3% of respondents), resistance to crop diseases (10.9%), drought tolerance (10.3%), resistance to crop insect pests (9.2%), higher seed yield (8.9%), higher thousand-seed weight (7.2%), higher oil content (6.3%), white seed colour (6.1%), early maturity (6.1%), and good oil qualities such as aroma and taste (5.7%). Therefore, there is a need for a dedicated sesame genetic improvement programme by integrating the above key production constraints and market- and farmer-preferred traits to develop and deploy new generation varieties to enhance the production, productivity, and adoption of sesame cultivars in Ethiopia.

Keywords: Ethiopia; market-preferred traits; participatory rural appraisal; production constraints; *Sesamum indicum*

2.1. Introduction

Sesame (*Sesamum indicum* L.) is an important oilseed crop valued in the food, feed, and cosmetics industries. The seed oil content of sesame is the highest (60%) when compared to other oilseed crops such as soybean (~20%), rapeseed (~40%), sunflower (~45%), and groundnut (45-56%) [1–5]. The seed oil is a rich source of protein (~24%), carbohydrate (~13.5%), vitamins (e.g., A and E), lignans (sesamin and sesamolin), and lipids [4,6–8]. Sesame seed has essential nutritional benefits to human health, including antioxidant, antiaging, antihypertensive, anticancer, and cholesterol-lowering properties. Further, the sesame oil seedcake contains about 32% crude protein (CP) and 8-10% oil serving as an essential feed for livestock and poultry [9]. The sesame biomass is used for animal feed, soap production, compost manure, and the production of potash, a cooking ingredient widely used in West African countries [10]. These and other benefits make sesame a highly valued economic crop globally [11].

Sesame is Ethiopia's second most crucial export crop after coffee (*Coffea arabica*). In 2020, the area allocated for sesame production was 375,119.95 ha, 45.7% of the estimated area under oil crop production [12]. It is an eminent crop and a significant contributor to the gross domestic product in Ethiopia [13]. Globally, a total of 2,211,339 tons of sesame grain was traded with a monetary value of 3.4 trillion USD in 2019 [14]. In 2019, sub-Saharan African countries exported about 1,465,493 tons of unprocessed sesame with a cash value of 1.9 trillion USD [14]. In 2019, Ethiopia's sesame export share was 8.96% of global exports, valuing 307 million USD [14]. In terms of global total sesame production, Ethiopia ranked ninth in 2019 with an annual production of 262,654 tons, after Sudan (1,210,000 tons), Myanmar (744,498 tons), India (689,310 tons), Tanzania (680,000 tons), Nigeria (480,000 tons), China (469,104 tons) and China Mainland (467,000 tons) [14].

In Ethiopia, sesame is mainly produced for household food and as a source of cash. It is predominantly grown by smallholders (95.5%) and medium-to-large commercial farmers (0.5%) under rainfed conditions [12]. Sesame production is primarily localized in the lowland areas of the country, where drought and heat stresses are common episodes. According to the Ethiopian Central Statistical Agency (CSA), during the 2019/2020 production seasos, the total area and volume of sesame production under medium-to-large commercial farming conditions was the highest in Tigray (56.42%), followed by Amhara (32.03%), Benishangul-Gumuz (7.25%), and Oromia (3.17%), whereas the total area and volume of production under smallholder farming systems was the highest in Amhara (51.82%), followed by Tigray (30.88%), Oromia (9.41%), and Benishangul-Gumuz (7.34%) [15].

The productivity of sesame is low and stagnant in Ethiopia and other major sesame growing regions in sub-Saharan Africa (<0.6 t/ha) because of many production constraints. The low yield of sesame is attributable to a lack of high-yielding and well-adapted varieties with little capsule shattering, the prevalence of biotic and abiotic stresses, and lack of modern production technologies such as optimal agronomic management practices, row planters, harvesters, and storage facilities [6,10,16–19]. Furthermore, Ethiopian farmers use landrace varieties of the crop that are inherently low yielders and prone to capsule shattering, leading to reduced productivity and low income. However, landraces are highly valued for having farmer-preferred attributes such as unique taste, aroma, and adaptation to grow under low-input farming systems and marginal agricultural lands. Consequently, these production constraints have yet to be systematically studied, prioritized, and documented in Ethiopia to guide research and development of the crop.

The sesame breeding research in Ethiopia was started in the late 1960s by the Ethiopian Institute of Agricultural Research (EIAR) based at the Melka Werer Agricultural Research Centre (WARC) [20]. From 1960 to 1979, some introduced landrace collections were used to initiate the sesame breeding programme in the country. The local sesame breeding pro- gramme has mainly focused on characterization and mass selection of landrace collections for desirable traits for direct recommendation and large-scale production, marketing, and breeding. For instance, a total of 32 improved sesame verities were developed and released by the EIAR through mass selection from among the local germplasm collections, introduction and crossing of selected parents [21]. The sesame varieties designated as Humera-1 and Setit-1 were released by the Humera Agri- cultural Research Centre (HuARC) in 2010. These varieties are widely grown by farmers for their early maturity, better yield performance, and broad adaptability. The yield performance of these varieties is low (<1.00 ton/ha), below the reportedly attainable yields of 2.53 and 1.62 tons/ha in Israel and China, respectively [14]. Therefore, sesame breeding programme in Ethiopia is required to select and identify desirable genotypes for practical breeding, genetic analyses, gene discovery, and developing high-performing and framer-preferred varieties. Sesame genetic improvement programmes should be guided by the prevalent production constraints of the growers as well as farmer- and market-preferred traits. These conditions will enable the development and deployment of new varieties according to the needs and preferences of the value chain, including participants such as farmers, traders, oil processors, and consumers.

Farmers are the main actors in agriculture enterprises, with a wealth of indigenous knowledge about their crops, farming systems, and constraints, and they have the means to adopt a technology [22]. Participatory rural appraisal (PRA) is a multidisciplinary research approach that aims to incorporate

knowledge and opinions of farmers in the planning and management of research development projects and programmes [23]. PRA studies have been conducted in Senegal and Mali to initiate sesame research programmes and develop policies that optimized sesame production and improved farmers' livelihoods [10]. Through a PRA study, Dossa et al. [10] identified a lack of marketing, a decline in soil fertility, limited access to land, drought stress, backward agricultural implements, lack of extension service, and limited access to agricultural inputs as most essential constraints to sesame production in Senegal and Mali. Myint et al. [24] reported insect pests, postharvest loss, drought, and salinity stresses as the overriding sesame production constraints in Myanmar. In Ethiopia, Abady et al. [25] used PRA tools. They found drought stress, poor soil fertility, poor supply of improved seed, preharvest diseases (e.g., root rots and leaf spots), low-yielding varieties, poor access to extension services, poor access to credit, and limited availability of improved varieties as key challenges for groundnut production.

Additionally, in Ethiopia, Sori [26] reported limited access to credit and scarcity of land as affecting the magnitude of groundnut supply to the marketplace. However, no recent study has documented farmers' perceptions of the production constraints on sesame and the preferred traits that farmers, market, and the value chain require in a new sesame variety in Ethiopia. Therefore, the objective of this study was to document sesame production opportunities and constraints and farmer- and market-preferred varieties and traits in eastern and southwestern Ethiopia as a guide for breeding and large-scale production. Consequently, this will serve as market research to guide variety design and development and to develop a successful marketing strategy.

2.2. Materials and Methods

2.2.1. Description of the Study Areas

The study was conducted in 2021 in two regions in Ethiopia: the Oromia region, in Babile and Gursum districts in eastern Ethiopia, and in the Southern Nations, Nationalities, and Peoples' (SNNP) region in Melekoza and Basketo districts. The study areas are among Ethiopia's major sesame growing belts (Table 2.1 and Figure 2.1). Babile and Gursum have a predominantly well-drained sandy loam soil that is ideal for sesame production. The rainfall distribution of the areas is bimodal, with the main rain (locally referred to as Meher rain) received during July to October and short rain (locally known as Belg rain) during March to May [27]. The mean annual maximum and minimum temperatures are 28.1 ^{o}C and 15.5 ^{o}C, respectively, with the total annual rainfall ranging from 507 to 984 mm in Babile district.

Gursum district receives an annual rainfall between 600 and 900 mm, with average temperatures varying between 25 and 33.5 °C. The predominant soil types of the Melekoza and Basketo districts are vertisol, ideal for sesame production. The rainfall distribution of the areas is bimodal, with the main rain received during July to October and short rain during March to May. The annual average rainfall is 750–1500 mm and 1000–1400 mm in Melekoza and Basketo districts, respectively, during the primary cropping season (June to August) [28]. During the study, the minimum and maximum temperatures in Melekoza district were 15.1 to 27.5 °C, while those in Basketo were 15 and 27 °C, respectively [28].

Table 2.1. Agroclimatic and soil characteristics of the study areas

Region	District	Villages	Altitude (Meter above Sea Level)	Geographic Coordinates	Annual Rainfall (mm)	Temperature (°C)	Soil Type
Oromia	Babile	Eibada Gemechu Ramata Salama	1642	9°13′09″ N, 42°19′25″ E	507–984	15.5–28.1	Sandy loam
	Gursum	Abadir Oda	1648	9°14′60.00″ N 42°14′60.00″ E	600–900	25–33.5	Chromic vertisol
SNNP	Melekoza	Salaysh Mender 01 Salaysh Mender 03	1395	6°29′59.99″ N, 36°39′59.99″ E	750–1500	15.1–27.5	Sandy loam
	Basketo	Angela 03 Angela 04	1600	6°15′0.00″ N, 36°34′59.99″ E	100–1400	15–27	Sandy loam

SNNP, Southern Nations, Nationalities, and Peoples'.

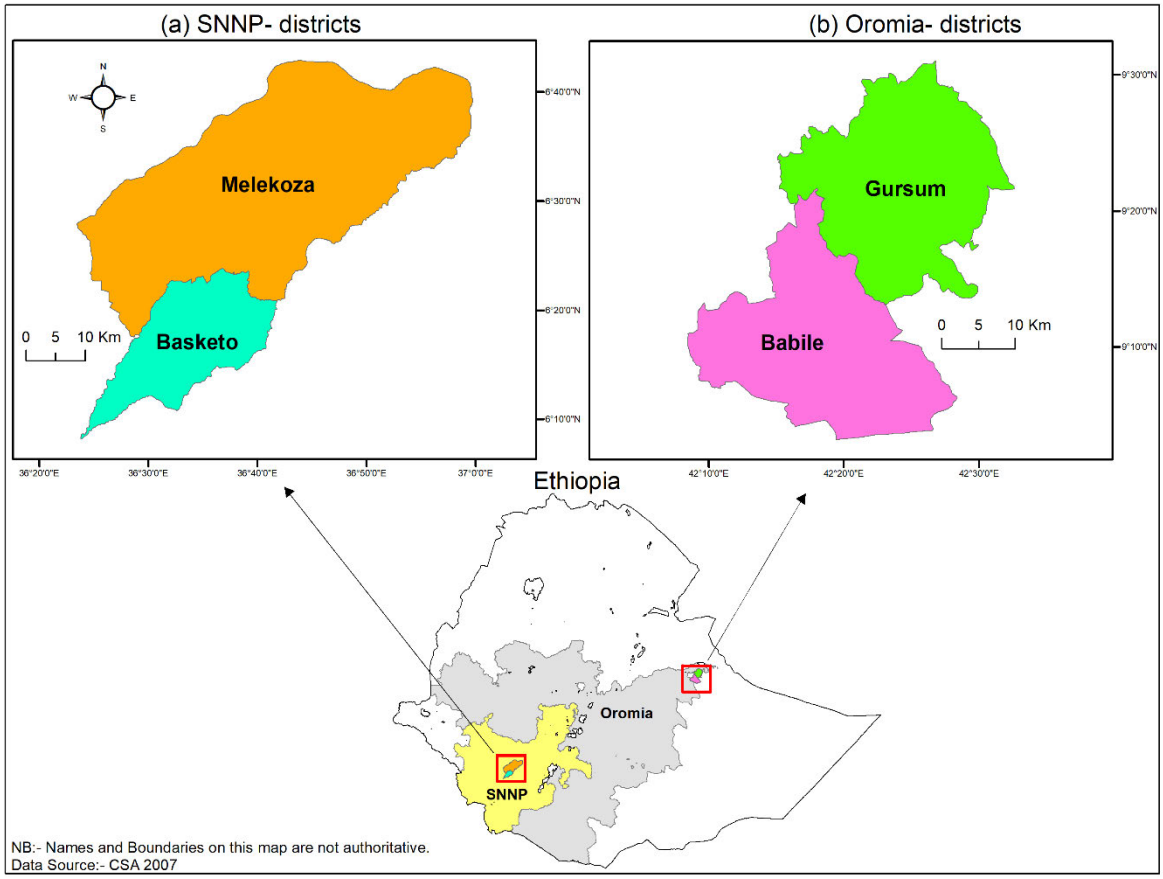

Figure 2.1. Map of Ethiopia showing the study sites shown with red squares: (a) SNNP districts (Melekoza and Basketo); (b) Oromia districts (Gursum and Babile). CSA, Central Statistical Agency; SNNPS, Southern Nations, Nationalities, and Peoples'.

2.2.2. Questionnaire Design and Sampling Procedures

A semi structured questionnaire and focused group discussions (FGDs) were used to collect data in the study areas. Data collected from FGDs were used to support and validate the information obtained from the semi structured questionnaire. A purposive sampling procedure was employed to select two sesame-growing regions, i.e., Oromia and SNNP, in Ethiopia. Figure 2.2 presents the sampling method, showing the selected regions, zones, districts, and villages for the study. From the Oromia region, two districts were selected from the east Hararghe zone (Babile and Gursum districts). In each district, two villages, locally referred to as 'kebeles', were subsampled, i.e., Eibada Gemechu and Ramata Salama from Babile and Abadir and Oda Oromia from Gursum districts. From the SNNP region, two districts were selected (Melokoza district from Gofa zone and Basketo special district).

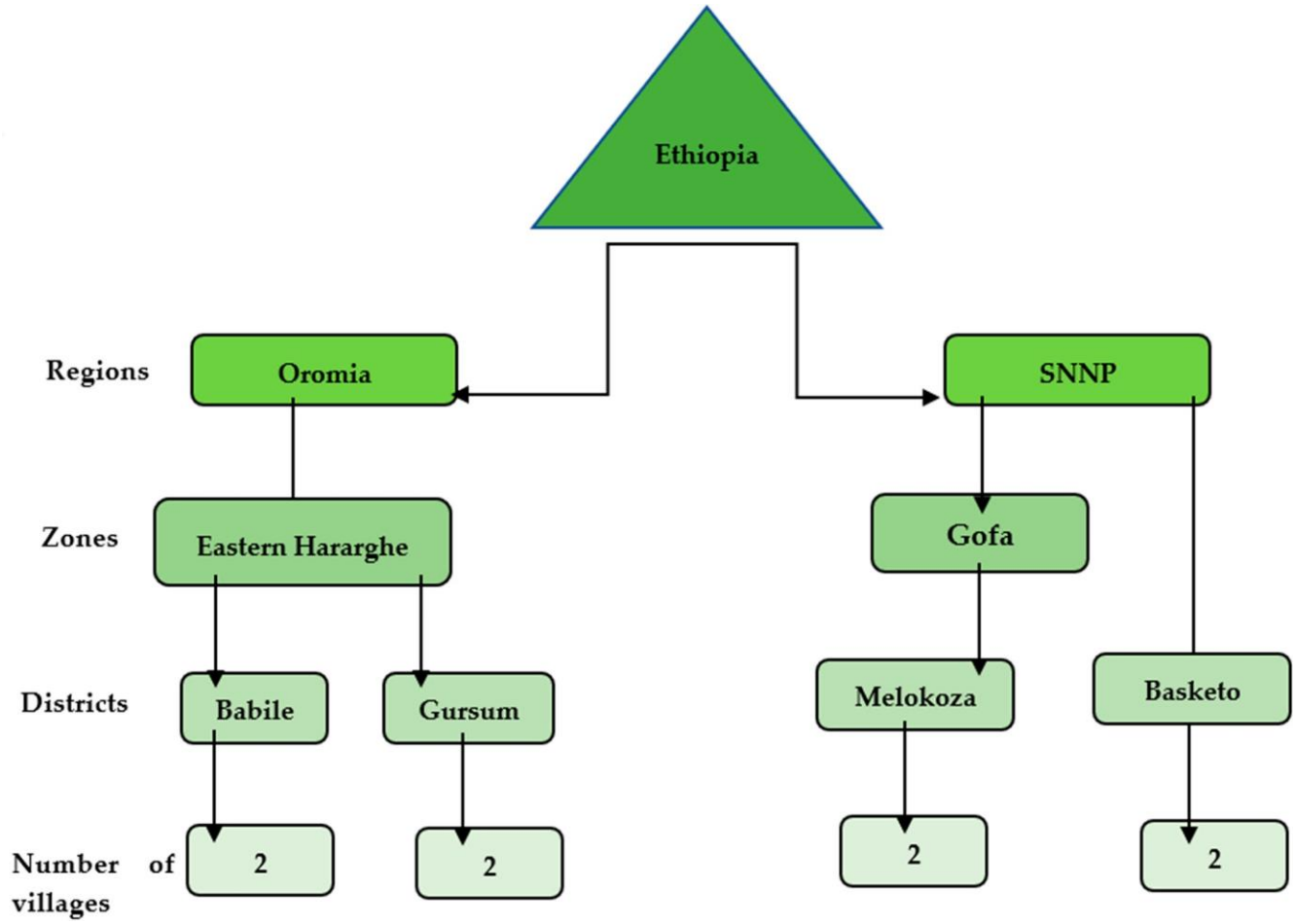

Figure 2.2. Sampling method cascading the selected regions, zones, districts, and villages for the study.

The farmers were selected with the assistance of the agricultural extension offices of the districts and villages. Based on the degree of homogeneity of the population, sesame production, and time and resource availability, 20 households from every eight villages were randomly sampled, providing a total of 160 respondents, of which only 14 (9%) were women-headed households (Table 2.2). FGDs were used to support and validate the interview data obtained from the semistructured questionnaire. The data collected from the FGDs included information on improved varieties, seed sources, seed and grain price, market information and challenges, and production constraints. The FGDs were held in four groups in six villages with 7 to 10 farmers per group across the four districts, except for Eibada Gemechu and Angela 03 villages in Babile and Basketo districts, respectively (Figure 2.3).

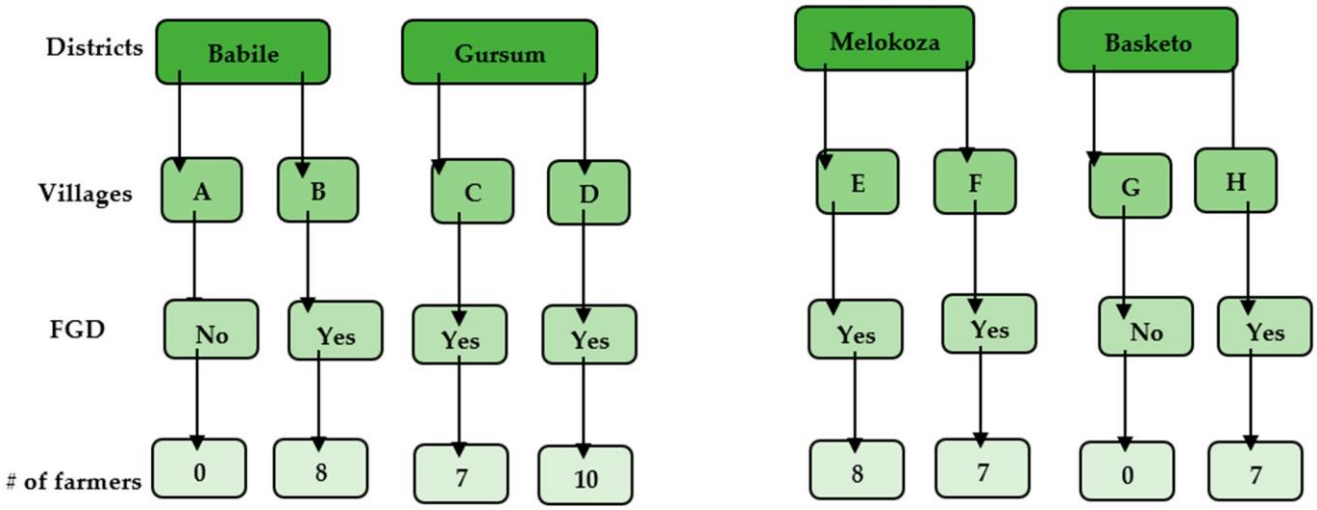

Figure 2.3. Sampling structure for the focused group discussions (FGDs) in study districts. Villages are denominated as follows: A, Eibada Gemechu; B, Ramata Salama; C, Abadir; D, Oda; E, Salaysh Mender 01; F, Salaysh Mender 03; G, Angela 03; and H, Angela 04.

2.2.3. Data Collection

Both primary and secondary data were collected. Primary data were collected through interviews using a semi structured questionnaire and focus group discussions. The responses of the selected farmers were based on their 2020 sesame farming experience. Enumerators were well-trained, and the questionnaires were pretested to ensure that enumerators understood the subject area, to create a clear awareness among the interviewees, and to improve the clarity of questions for proper data collection. Data were collected through the semi structured questionnaire on demographic characteristics of the households, farm sizes, the farming systems used, the sesame area cultivated and the productivity status thereof, seed systems, production constraints, important crop traits preferred by farmers, and market accessibility. Secondary data, such as area coverage, production, and productivity of the crop, were collected from the respective districts of agricultural offices used in the study.

2.2.4. Data Analysis

Both quantitative and qualitative data were coded and subjected to analysis using the Statistical Package for the Social Sciences (SPSS) software version 20 [29]. The quantitative data were coded before analysis. Descriptive statistics, such as frequencies and percentages, were calculated. In addition, chi-square and t-tests were performed using the cross-tabulation procedure of SPSS.

2.3. Results

2.3.1. Sociodemographic Description of Respondent Farmers

Out of 160 smallholder farmers were interviewed individually, 46 participated in focus group discussions. Table 2.2 shows the demographic characteristics of the participants. Of the 160 smallholder farmers interviewed, 91% and 9% were males and females, respectively. Gursum district had a relatively higher number of male respondents (97.5%), followed by Babile and Melokoza districts (92.5% each), while Basketo district had 82.5% male participants.

Seventy percent of the households had a family size of 6 to 10 individuals, while 24% had 2 to 5 individuals. Polygamy is a common culture in the study areas and is linked with the high population growth rate in the rural areas. The majority (73%) of the households were between 30 and 50 years of age, indicating that the middle-aged adult farmers were highly engaged in sesame production in the study areas. Nineteen percent of the participants ranged from 18 to 29 years of age, while eight percent were between 51 and 65 years of age. About 49% of the respondents had attended primary school, while 30% of the participants could not read and write. The rest of the respondents were able to read and write (16%) or had attended secondary school (4%) and college (0.6%) (n = 160).

Table 2.2. Social and demographic information of respondent farmers in the study areas (%; n = 160).

Variable	Class	Districts				%Mean	*df	Chi-Square	*p*-Value
		Babile	Basketo	Gursum	Melokoza				
Gender	Female	7.5	17.5	2.5	7.5	9.00	3	5.949	0.114
	Male	92.5	82.5	97.5	92.5	91.00			
Family size	2–5	25.0	22.5	25.0	22.5	23.75	6	1.377	0.967
	6–10	67.5	70.0	72.5	70.0	70.00			
	11–15	7.5	7.5	2.5	7.5	6.25			
Age (years)	18–29	17.5	20.0	27.5	12.5	19.38	6	4.162	0.655
	30–50	72.5	75.0	62.5	80.0	72.50			
	52–65	10.0	5.0	10.0	7.5	8.13			
Education level	Illiterate	42.5	37.5	27.5	12.5	30.00	12	20.319	0.061
	Read and write	20.0	15.0	20.0	10.0	16.25			
	Primary school (Grade 2 to 8)	35.0	42.5	50.0	67.5	48.75			
	Secondary school (Grade 9 to 12)	2.5	2.5	2.5	10.0	4.38			
	College	0.0	2.5	0.0	0.0	0.63			

* df, degrees of freedom.

2.3.2. Main Socioeconomic Activities in the Study Districts

Off-Farm Income Sources of Farmers

Table 2.3 presents the off-farm income sources of the respondent farmers. The result showed that about 4% of the respondents earned off-farm income, most frequently through a daily labourer wage and trading, followed by carpentry, serving in churches, construction sectors, and pensions. The majority (88%) of the households did not have off-farm income sources except for crop production and livestock rearing.

Table 2.3. Off-farm income sources of participants in the study areas (%; n = 160).

Income Sources	Districts				%Mean	*df	Chi-Square	*p*-Value
	Babile	Basketo	Gursum	Melokoza				
None	95.0	78.0	90.0	88.0	87.500	18	23.410	0.175
Daily labour	5.0	5.0	5.0	0.0	3.750			
Trader	0.0	5.0	0.0	10.0	3.750			
Builder	0.0	0.0	3.0	0.0	0.625			
Carpenter	0.0	5.0	3.0	0.0	1.875			
Church employ	0.0	5.0	0.0	3.0	1.875			
Pension	0.0	3.0	0.0	0.0	0.625			

*df, degrees of freedom.

2.3.3. Farmers' Awareness of Sesame Varieties

About 62% of the farmers had information regarding improved sesame varieties, via development agents (34%) and local radio programmes (25%). About 38% of participant farmers were not aware of the improved varieties (Table 2.4). The following sesame varieties were known and cultivated in the study areas: Humera-1, Abasena, Wollega, Adi, and unnamed. There was a highly significant difference ($p < 0.00$; $\chi 2 = 158.71$) in the sesame varieties cultivated among the four districts. The majority (56%) of the farmers cultivated unnamed sesame varieties. About 38% of respondent farmers sourced sesame seeds from neighbours through farmer-to-farmer exchange and farm-saved seeds, while 19% of respondents sourced from local markets and 6% from nongovernment organizations such as Self Help, the Hararghe Catholic Secretariats (HCS), and the Catholic Relief Service (CRS) (Table 2.4). There were no government-linked sesame seed enterprises or cooperative seed production in the study areas. Also, there were no formal seed systems to support sesame production in the study areas except those provided by nongovernment organizations, which provided seeds for demonstration purposes 10 years ago. In Melokoza and Basketo districts, 30% of the respondent farmers reported

using sesame variety Humera-1, acquired through farmer- to-farmer exchange, farm saving, or the local market. During the FGDs, participants stated that Humera-1 was their chosen variety for its white seed colour, high oil content, and yield potential, fetching reasonable market price compared to other cultivars in the study areas. Sixty percent of the respondent farmers reported participating in technology transfer activities. About 38%, 14%, and 8% of farmers participated in farmer training centres (FTC), on-farm trials, and farmers' field days, respectivel thereby received technical backstopping in sesame production (Table 2.4). About 38% of the respondent farmers reported that FTCs were the main sources of information and technology transfer methods.

2.3.4. Sesame Cropping System and Production Status

Sesame cropping systems and perceptions of production trends in the four districts are summarized in Table 2.5. There was a highly significant difference ($p < 0.00$; $\chi 2 = 108.542$) in sesame cropping systems among the four districts. Sixty percent of the farmers cultivated sesame as a sole crop, while 40% intercropped sesame with sorghum (37.5%), groundnut (8.75%), or maize (3.75%) (n = 160) (Figure 2.4). Most of the respondent farmers (41.88%) in the study areas practiced crop rotation of sesame with maize, followed by mung bean (18.75%), haricot bean (17.50%), and groundnut (9.38%). Crops and their areas of production in the study areas are summarized in Figure 2.5. Sesame had the most significant area coverage, followed by maize and sorghum (Figure 2.5). During the FGDs, the majority (46%) of the households reported the trend that sesame production areas had decreased, while 34% and 19% reported increasing and constant trends, respectively, for the last five years. The decreased area of sesame production was mainly attributed to a lack of improved varieties, abiotic and biotic stresses, and a lack of extension services and market linkage.

Table 2.4. Farmers' awareness about improved sesame varieties, seed sources, and participation in technology transfer activities in the study areas (%; n = 160).

Variable	Category	Districts				%Mean	*df	Chi-Square	*p*-Value
		Babile	Basketo	Gursum	Melokoza				
Seed source	Local market	22.5	10.0	40.0	2.5	18.75	9	84.000	0.000
	NGOs	12.5	12.5	0.0	0.0	6.25			
	Farmer to farmer	50.0	2.5	57.5	40.0	37.50			
	Farm saved	15.0	75.0	2.5	57.5	37.50			
Information about improved varieties	Yes	25.0	80.0	45.0	97.5	61.88	3	54.976	0.000
	No	75.0	20.0	55.0	2.5	38.13			
Source of information	No	75.0	20.0	55.0	2.5	38.13	9	97.809	0.000
	Local radio programme	20.0	17.5	37.5	25.0	25.00			
	Developmental agent	0.0	62.5	0.0	72.5	33.75			
	Farmer to farmer	5.0	0.0	7.5	0.0	3.13			
Varieties grown	Unnamed	100.0	57.5	65.0	2.5	56.25	12	158.711	0.00
	Humera-1	0.0	35.0	0.0	85.0	30.00			
	Abasena	0.0	5.0	0.0	0.0	1.25			
	Wollega	0.0	2.5	0.0	12.5	3.75			
	Adi	0.0	0.0	35.0	0.0	8.75			
Participation in technology transfer	Yes	35.0	80.0	35.0	90.0	60.00	3	42.500	0.000
	No	65.0	20.0	65.0	10.0	40.00			
Methods of technology transfer	No	65.0	20.0	65.0	10.0	40.00	9	67.323	0.000
	On-farm trials	20.0	7.5	22.5	7.5	14.38			
	Field days	2.5.0	10.0	2.5	15.0	7.50			
	FTC	12.5	62.5	10.0	67.5	38.13			

NGOs, nongovernment organizations; *df, degrees of freedom, FTC, farmer training centre.

Table 2.5. Farmers' sesame cropping systems and perceptions of production trends in the study areas (%; n = 160).

Variable	Category	Districts				%Mean	*df	Chi-Square	*p*-Value
		Babile	Basketo	Gursum	Melokoza				
Cropping system	Sole cropping	12.5	100.0	27.5	100.0	60.00	3	108.542	0.000
	Inter cropping	87.5	0.0	72.5	0.0	40.00			
Sesame intercropping with	Sorghum	80.0	0.0	28.0	0.0	37.50	9	162.819	0.000
	Groundnut	12.5	0.0	9.0	0.0	8.75			
	Maize	7.5	0.0	3.0	0.0	3.75			
	NA	0.0	100.0	0.0	100.0	50.00			
Sesame rotation with	Maize	17.5	42.5	40.0	67.5	41.88	12	98.490	0.000
	Mung bean	0.0	45.0	0.0	30.0	18.75			
	Haricot bean	25.0	10.0	35.0	0.0	17.50			
	Groundnut	25.0	0.0	12.5	0.0	9.38			
	None	32.5	2.5	12.5	2.5	12.50			
Sesame production status in the last 5 years	Constant	17.5	25.0	22.5	9.0	19.38	6	40.809	0.000
	Increasing	17.5	47.5	60.0	24	34.38			
	Decreasing	65.0	27.5	17.5	7.0	46.25			

* df, degrees of freedom; NA, not applicable.

Figure 2.4. (A)—a sole crop of sesame; (B)—sesame intercropped with sorghum in Babile district; (C)—white seeded sesame preferred mainly by farmers (photos by Desawi H.).

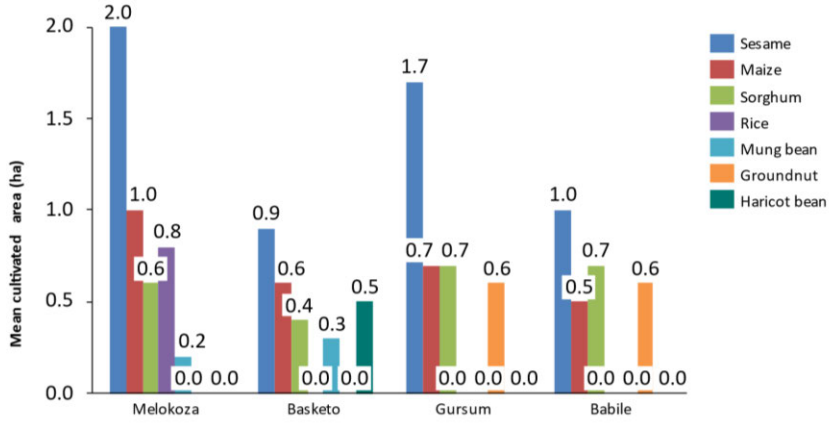

Figure 2.5. Mean cultivated area (ha) allocated for different crops grown in the study districts in the 2020/2021 cropping season.

2.3.5. Constraints to Sesame Production in Ethiopia

Farmers identified the major abiotic, biotic, and market constraints affecting sesame production in the study areas (Table 2.6). There was a highly significant difference ($p < 0.00$; $\chi 2 = 30.204$) among districts' access to improved seeds. Farmers' perceptions in regard to the assessed production constraints were further explored through FGDs. About 83% of households reported that lack of access to improved seeds ranked as a leading constraint of sesame production. This was mainly attributed to the lack of government- linked sesame seed enterprises and cooperative seed production in the study areas. About 87.5 to 90% of the households reported low yield by the existing varieties as the second most crucial yield-limiting factor in the study areas.

2.3.6. Market-Preferred Traits of Sesame

Farmers identified white seed colour, large seed size, true-to-type seed, high oil content, and increased thousand-seed weight as the most critical sesame market-preferred traits in the study areas (Table 2.7). Farmers in all the districts ranked true-to-type seed as the first market-preferred trait, followed by white seed colour and high oil content ($n = 160$). During the FGDs, farmers stated that the middlemen and district trade offices mysteriously engaged in market price fixing without farmers involvement. This indicates that there are no price regulations favouring the framer's involvement in sesame market systems in the study areas. Therefore, it is essential for the Ministry of Trade and Industry (MoTI) through the Ethiopian Commodity Exchange (ECX) to strengthen sesame marketing regulations and to avail farmers of market information.

Table 2.6. Percentage of farmers reported sesame production constraints in the study areas.

Traits	Rank	Districts				%Mean	*df	Chi-Square	*p*-Value
		Babile	Basketo	Gursum	Melekoza				
Lack of access to improved seed	1st	95.0	75.0	97.5	62.5	82.50	9	30.204	0.000
	2nd	0.0	12.5	2.5	27.5	10.60			
	3rd	2.5	0.0	0.0	2.5	1.30			
	4th	2.5	12.5	0.0	7.5	5.60			
High cost of seed	1st	65.0	37.5	80.0	17.5	50.00	9	52.019	0.000
	2nd	7.5	20.0	12.5	25.0	16.25			
	3rd	25.0	10.0	0.0	30.0	16.25			
	4th	2.5	32.5	7.5	27.5	17.50			
Low-quality seed	1st	37.5	50.0	55.0	25.0	41.88	9	36.955	0.000
	2nd	7.5	20.0	10.0	45.0	20.63			
	3rd	30.0	0.0	20.0	15.0	16.25			
	4th	25.0	30.0	15.0	15.0	21.25			
Low yield	1st	90.0	70.0	87.5	47.5	73.75	9	32.538	0.000
	2nd	2.5	12.5	5.0	30.0	12.5			
	3rd	5.0	2.5	2.5	15.0	6.25			
	4th	2.5	15.0	5.0	7.5	7.5.0			
Climate change (Drought)	1st	52.5	32.5	42.5	32.5	40.00	9	16.394	0.059
	2nd	12.5	45.0	20.0	30.0	26.88			
	3rd	25.0	10.0	17.5	25.0	19.38			
	4th	10.0	12.5	20.0	12.5	13.75			
Insect pests	1st	57.5	80.0	52.5	47.5	59.38	9	28.654	0.001
	2nd	5.0	2.5	7.5	30.0	11.25			
	3rd	15.0	10.0	15.0	15.0	13.75			
	4th	22.5	7.5	25.0	7.5	15.63			
Diseases	1st	77.5	82.5	65	52.5	69.38	9	8.726	0.001
	2nd	0.0	7.5	12.5	30.0	12.50			
	3rd	17.5	2.5	22.5	10.0	13.13			
	4th	5.0	7.5	0.0	7.5	5.00			
Weeds	1st	32.5	50.0	42.5	65.0	47.50	9	25.715	0.002
	2nd	7.5	25.0	20.0	22.5	18.75			
	3rd	27.5	10.0	25.0	5.0	16.88			
	4th	32.5	15.0	12.5	7.5	16.88			
Lack of market information	1st	42.5	87.5	30.0	60.0	55.00	9	37.449	0.000
	2nd	5.0	5.0	15.0	17.5	10.63			
	3rd	25.0	2.5	27.5	12.5	16.88			
	4th	27.5	5.0	27.5	10.0	17.50			
Low market price	1st	57.5	90.0	62.5	65.0	68.75	9	15.972	0.067
	2nd	12.5	5.0	12.5	15.0	11.25			
	3rd	12.5	5.0	17.5	12.5	11.88			
	4th	17.5	0.0	7.5	7.5	8.13			

* df, degrees of freedom

Table 2.7. Percentages of farmers reporting sesame market-preferred traits in the study areas.

Traits	Rank	Districts				%Mean	*df	Chi-Square	*p*-Value
		Babile	Basketo	Gursum	Melekoza				
White seed colour	1st	27.5	37.5	17.5	32.5	28.75	12	25.624	0.012
	2nd	15.0	5.0	10.0	17.5	11.88			
	3rd	15.0	42.5	17.5	20.0	23.75			
	4th	20..0	12.5	30.0	17.5	20.00			
	5th	22.5	2.5	25.0	12.5	15.63			
Large seed size	1st	10.0	0.0	12.5	5.0	6.88	12	29.69	0.003
	2nd	25.0	7.5	37.5	20.0	22.50			
	3rd	27.5	15.0	15.0	27.5	21.25			
	4th	25.0	55.0	32.5	27.5	35.00			
	5th	12.5	22.5	2.5	20.0	14.38			
True-to-type seed	1st	12.5	45.0	25.0	62.5	36.25	12	65.989	0.000
	2nd	27.5	22.5	20.0	22.5	23.13			
	3rd	27.5	30.0	35.0	10.0	25.63			
	4th	22.5	2.5	7.5	5.0	9.38			
	5th	10.0	0.0	12.5	0.0	5.63			
High oil content	1st	52.5	2.5	35.0	5.0	23.75	12	65.989	0.000
	2nd	2.5	0.0	15.0	17.5	8.75			
	3rd	7.5	12.5	7.5	25.0	13.13			
	4th	25.0	27.5	27.5	30.0	27.50			
	5th	12.5	57.5	15.0	22.5	26.88			
High 1000-seed weight	1st	10.0	20.0	5.0	0.0	8.75	12	72.709	0.00
	2nd	12.5	67.5	7.5	32.5	30.00			
	3rd	37.5	5.0	30.0	37.5	27.50			
	4th	10.0	2.5	22.5	22.5	14.38			
	5th	30.0	5.0.	35.0	7.5	19.38			

* df, degrees of freedom.

2.3.7. Farmer-Preferred Traits

In the present study, farmers selected sesame cultivars for production based on reasonable market price, resistance to diseases, drought tolerance, resistance to insect pests, high yield, high 1000 seed weight, high oil content, and white seed colour, in that order (Table 2.8). During the FGDs, farmers described reasonable market price, high oil content, white seed colour, and high thousand-seed weight as market-preferred traits. These attributes attracted premium prices for sesame farmers.

Table 2.8. Farmer-preferred traits (% farmers) in sesame varieties in the study areas.

Traits	Districts				Mean
	Babile	Basketo	Gursum	Melekoza	
Yield potential	11.45	7.04	11.24	5.89	8.91
High oil content	9.63	3.45	8.73	3.52	6.33
High oil quality (e.g., aroma and taste)	6.97	4.21	6.98	4.74	5.73
Good market price	12.64	9.82	12.82	10.00	11.32
White seed colour	5.80	5.47	6.56	6.66	6.12
High 1000-seed weight	6.56	7.66	7.03	7.61	7.22
Tall plant height	5.44	1.43	5.31	2.69	3.72
High number of capsules	2.47	5.56	2.62	5.39	4.01
High number of branches per plant	5.70	6.47	4.73	4.89	5.45
Resistance to insect pests	7.26	10.65	7.24	11.52	9.17
Resistance to disease	9.42	12.19	9.31	12.60	10.88
Drought tolerance	9.00	11.06	8.94	12.37	10.34
Early maturity	3.95	10.57	4.47	5.41	6.10

2.4. Discussion

2.4.1. Sociodemographic Description of Respondent Farmers

The participation of male (91%) farmers was higher than female (9%) farmers in sesame production in the study areas (Table 2.2). The disparity indicates that male households dominated sesame production and women are highly influenced by economic, cultural and religious factors in the study areas. In agreement with this finding, Dossa et al. [10] reported that more male than female farmers were involved in sesame production in Senegal and Mali. Gender imbalance also occurred in the findings of Abady et al. [25] on groundnut production in similar study areas of Ethiopia.

Most of the households had a family size of 6 to 10 individuals (Table 2.2). In Ethiopia, sesame production relies on traditional production technologies that result increased production cost. And increased family members can contribute more to the farm activities. Mendola [30] reported that the household is a significant source of labour in smallholder farming communities, suggesting that the larger the household size, the greater the labour force available to operate farming activities and minimize the cost of production.

The majority of the households were between 30 and 50 years of age (Table 2.2). It is believed that this active age group plays a crucial role in decision-making, improving the local economy, adopting improved technologies, and conducting farm operations. Previously, these demographic groups were reported in the sesame production areas in Senegal and Mali [10].

About 49% of the respondents had attended primary school, followed by 30% who were unable to read and write. A household's education level has a great impact on the management of the family's livelihood and active participation in decision making, adoption of technologies, and farm operations [31]. Farmers with no formal education are unwilling to adopt improved technologies and extension services and rely on traditional farming practices in Burkina Faso [32]. Therefore, enrolling children in the local schools is most vital to help households adopt improved technologies, extension services, and access to information that will improve the production and productivity of the crop, thereby improving the livelihood of the family. Further, developmental agents or any service providers need to use vernacular language during the implementation of technology transfer activities. Local language would improve the level of understanding of illiterate households towards adopting improved technologies in the study areas.

2.4.2. Main Socioeconomic Activities in the Study Districts

Off-Farm Income Sources of Farmers

Most of the studied households did not have off-farm income sources (Table 2.3). Typically, the income sources of households in developing countries are dependent on agriculture, as favoured by the agricultural-led industrialization policies of said countries. Abady et al. [25] and Daudi et al. [33] reported that most of groundnut farmers' livelihoods were derived from agriculture in Ethiopia and Tanzania, even more so in the former. Different sources of income for farmers can help ensure their livelihoods. Therefore, it is essential to design and introduce projects to diversify farmer's portfolios of income sources in the study areas to mitigate the impacts of crop failure and livestock death due to abiotic and biotic stresses.

2.4.3. Farmers' Awareness of Sesame Varieties

The study showed that most farmers had information about improved varieties through development agents (extension workers at village level), radio programmes, and farmer-to-farmer information exchange in the study areas (Table 2.4). Even though the farmers had information, they cultivated varieties often sourced from the informal seed system in the study areas. The farmers cultivated improved sesame varieties, but there were no government-linked sesame seed enterprises or cooperative seed production in the study areas. Thirty percent of the respondents in Melokoza and Basketo districts adopted the Humera-1 variety, developed and released by Humera Agricultural Research Centre (HuARC) in 2010. This variety was developed through mass selection from among

local germplasm collections. Most farmers in Ethiopia and Tanzania used seed of groundnut landraces for multiple benefits such as good oil quality, grain yield, adaptability to environmental stresses, drought tolerance, seed availability, and the ability to adapt to adverse climatic conditions and to retain seeds for the next cycle of planting [25,33]. The study areas' farmers classified Humera-1 and Adi as early-maturing cultivars and Abasena and Wollega as medium-maturing cultivars with relatively better bacterial blight resistance. High thousand-seed weight, high oil content, and white seed colour are among the most essential traits considered in the export standards of sesame [34]. The findings of this study show the need to design and introduce government-assisted sesame seed enterprises and cooperative seed production and to strengthen the extension service delivery system to enhance the dissemination and adoption of improved sesame agricultural technologies and enhance the livelihoods of the farmers in the study areas.

Most of the respondent farmers (60%) participated in technology transfer activities in the study areas. Chi-square analysis revealed the presence of highly significant differences among the four districts in methods of technology transfer ($p < 0.000$; $\chi 2 = 67.323$) (Table 2.5). The majority of the farmers reported that FTCs were among the main information and technology dissemination centres through demonstrations of methods to the farmers. Therefore, demonstrating improved varieties with the full package of agronomic practices through on-farm trials and FTCs, strengthening the extension services, and increasing availability of sesame seeds through engaging government seed enterprises and private seed producers would boost sesame production and productivity in the study areas.

2.4.4. Sesame Cropping System and Production Status

Most respondent farmers in the study areas grew sesame as a sole crop (Table 2.5). Farmers in Babile and Gursum districts intercropped sesame with sorghum, maize, and groundnut crops to diversify their cash income and mitigate the adverse effects of crop failure associated with growing sesame as a sole crop. In line with the current findings, Mesfin et al. [35] reported sesame intercropping with sorghum and millet in the study areas. Some farmers practiced sesame intercropping with sorghum and millet crops in Senegal and Mali [10]. Furthermore, Mkamilo [36] reported sesame intercropping with maize in southeast Tanzania.

The present study revealed that most of the farmers practiced sesame rotation with maize, mung bean, haricot bean, and groundnut, mainly to restore soil fertility and reduce pest pressure in the four

districts (Table 2.5). Conversely, most interviewees practiced monoculture of sesame in Senegal and Mali [10]. The majority of the farmers explained that the trend of sesame production in the study areas was decreasing, mainly attributing this trend to lack of improved varieties, abiotic and biotic stresses, a lack of better agronomic practices, and poor extension services and market linkages in the study areas. This result corroborated the findings of Abady et al. [25] in regard to groundnut production in the same study areas in Ethiopia. The findings of this study show the need to design and introduce government-assisted sesame seed enterprises and cooperative seed production and strengthen the extension service delivery system to enhance the dissemination and adoption of improved sesame agricultural technologies and enhance the livelihoods of the farmers in the study areas.

2.4.5. Constraints to Sesame Production in Ethiopia

Farmers identified lack of access to improved varieties, high cost of seeds, low quality of seeds, low yield, climate change, insect pests, diseases, weeds, lack of market information, and low market price as the most critical constraints affecting sesame production (Table 2.6). Most households reported a lack of access to improved seeds as the most crucial constraint on sesame production due to the lack of a formal seed sector. The majority of the farmers identified low yield as the second most important constraint in sesame production in the study areas, suggesting that farmers grow unimproved varieties often sourced from the informal sector. Similarly, Teklu et al. [37] reported that the low productivity of sesame was due to a lack of improved and high-yielding varieties, traditional production technologies, and abiotic and biotic stresses, among other constraints. The authors also reported that landrace varieties were the primary sources of seed for cultivating the crop in Ethiopia. The low yield of sesame in SSA is mainly attributable to a lack of high-yielding, well-adapted varieties and shattering-tolerant cultivars, the prevalence of biotic and abiotic stresses, and the use of traditional production and harvesting systems [6,10,16–19,37,38]. The respondent farmers also identified low-quality and expensive sesame seeds sourced from the local market as one of the important production constraints. For instance, farmers in Basketo and Melokoza districts bought 1 kg of improved seed with a monetary value of 60 Birr (1.30 USD). Farmers expressed poor seed systems and lack of quality seed producers as a bottleneck in sesame production.

Climate change and insect pests are among the most essential yield-limiting factors mentioned by the sesame growers in the study areas (Table 2.6). Insect pests primarily cause yield reduction. A mean yield loss of 25% has been reported due to insect pest attacks [39]. Similarly, Myint et al. [24] reported that drought and insect pests were among the major sesame production constraints in Myanmar.

Therefore, the development and introduction of drought-tolerant and insect pest-resistant varieties to the seed system is crucial to minimize the risk of crop failure due to abiotic and biotic stresses and increase the crop's productivity.

Furthermore, farmers in the study areas mentioned a lack of market information and low market price as among the most critical challenges in sesame production. In the study areas, there were no market infrastructures or market information delivery systems, and the growers were forced to sell their produce at a lower price. The market value chain is not well-developed, which often highly discourages farmers from producing the crop. For instance, farmers in Basketo and Melokoza districts sold 100 kg of their products with 1000–3000 Birr (about 22.3–67 USD) at farm gate price but at 3000–3500 Birr (about 67–78 USD) during the study period. The lack of an encouraging production environment in the study areas highly affected farmers and discouraged them from producing the crop in a larger quantity and with better quality. In agreement with the current findings, Myint et al. [24] reported that lower production and productivity in some areas of Myanmar was due to a lack of market for the farmers. There is a need to improve the sesame value chain through incorporating improved and high-yielding varieties into the formal seed system, more expansive use of the best agronomic practices, strengthening the extension services, and developing market infrastructure and on-time market information delivery. These attributes can motivate farmers to produce higher quantities of better-quality seed to the market.

2.4.6. Market-Preferred Traits of Sesame

Sesame is an important oilseed crop serving various value chains globally. In the present study, farmers identified white seed colour, higher seed size, true-to-type seed, higher oil content, and increased thousand-seed weight as the most crucial sesame market preferred traits (Table 2.7). Farmers ranked true-to-type seed as the first most crucial market-preferred trait, followed by white seed colour and higher oil content. Higher thousand-seed weight, higher oil content, and white seed colour are among the most critical traits considered in the export standards of sesame [34]. Therefore, introducing improved, higher-yielding, higher-thousand-seed weight, higher-oil content, and white seeded sesame varieties is considerably important to increasing the crop's market value.

2.4.7. Farmer-Preferred Traits

Farmers identified reasonable market price, resistance to disease, drought tolerance, resistance to insect pests, high yield, high 1000-seed weight, high oil content, and white seed colour as the most important traits in the study areas (Table 2.8). The ultimate goal of farmers when engaging in crop production is to increase productivity and obtain better income from the market, thereby improving their livelihood. Farmers in the study areas suggested that varieties with high yield, drought tolerance, and insect pest and disease resistance were highly preferred. These varieties avert the risks of crop failure due to abiotic and biotic stresses.

2.5. Conclusions

Farmers identified limited access to improved seeds as the most critical production constraint, followed by low yield, diseases, and low market price. Other production constraints included insect pests, lack of market information, and high cost of seed. These constraints were attributable to the absence of a dedicated breeding programme, lack of a formal seed sector, poor extension services, and underdeveloped pre- and postharvest infrastructures. The essential market-preferred traits of sesame included true-to-type seed, white seed colour, and high seed oil content. The vital farmer-preferred attributes included reasonable market price, resistance to crop diseases, drought tolerance, resistance to crop insect pests, high seed yield, high thousand-seed weight, high oil content, white seed colour, early maturity, and good oil quality in areas such as aroma and taste. Therefore, there is a need for a strong sesame genetic improvement programme that would integrate the above key production constraints and market- and farmer-preferred traits to develop and deploy new vrieties to enhance stable production, productivity, and adoption of sesame cultivars in Ethiopia.

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Chapter 3. Genetic variability and association of yield-related traits in sesame

Abstract

The extent of phenotypic variation among sesame germplasm influences the expression of economic traits and the response to selection. The objectives of this study were to determine the variance components, broad-sense heritability (h^2b) and association of seed and oil yield-related traits in Ethiopian sesame (*Sesamum indicum* L.) germplasm to guide breeding. One hundred sesame germplasm were evaluated under field conditions in two locations using a 10 x 10 lattice design with two replications. The findings revealed higher genotypic coefficient of variation and h^2b, for number of secondary branches (NSB), and seed yield per hectare (SYH) suggesting high genetic gains can be achieved through selection. Seed yield was significantly correlated with oil yield (r = 0.57), number of seeds per capsule (r = 0.42), thousand seed weight (r = 0.31), number of capsules per plant (r = 0.29), and oil content (r = 0.25). Higher direct effects of OYH and number of seeds per capsule (NSPC) were recorded on SYH, while SYH, number of capsules per plant (NCPP) and TSW had a higher direct effect on OYH. The genotypes Hirhir Kebabo Hairless-9, Setit-3, Orofalc ACC-2, Hirhir Humera Sel-6, Setit-1 and ACC-NS-007(2) were found superior and the selected genotypes should be promoted for further field stability evaluation, which might be utilized in future sesame breeding programmes and production.

Keywords: Correlation, genetic advance, heritability, path analysis, *Sesamum indicum*

3.1. Introduction

Sesame (*Sesamum indicum* L.) is a predominantly self-pollinating crop with <1% cross-pollination (Ashri, 2007). It is a relatively drought-tolerant crop thriving under water-limited agro-ecologies where most crops fail (Ashri, 2007). Ethiopia and India are believed to be the centres of genetic diversity of sesame (Bedigian, 1981; Seegeler, 1983).

Sesame is an important seed oil crop serving diverse value chains. It is useful in the food, feed, confectionery and baking industry. Sesame seed is industrially processed to supply cooking oil, tahini, halvah and cosmetic oils, while the seed cake is used for livestock feed. The seed oil content of sesame varies from 41% to 60%, the highest value compared to other seed oil crops (De la Vega et al. 2002; Zheljazkov et al. 2008; Wei et al. 2013;Dossa et al. 2018). Sesame oil comprises of the following fatty acids: linoleic acid (~46%), oleic acid (~38%), palmitic acid (~12%) and stearic acid (~4%) (Uzun et al. 2002 and Uzun et al., 2008; Anastasi et al. 2017; Dossa et al. 2018). Sesame seed is a rich source of protein (≈24%), vitamins (e.g. A and E), lignans (sesamin and sesamolin), γ-tocopherol, phytosterols (β-sitosterol and Campesterol), policosanols (Docosanol, Tetracosanol, Hexacosanol and Octacosanol) and lipids (Were et al. 2006; Anastasi et al. 2017; Gharby et al. 2017; Dossa et al. 2018). Due to the above favourable fatty acid and nutrient profiles, sesame oil is valued for human diet and in the pharmaceutical industry.

Sesame is an important crop in sub-Saharan Africa (SSA) including Ethiopia. It is the leading seed oil crop in Ethiopia, occupying 39.4% of the total production area allotted to oil crops. In 2018, sesame production area was 294,819.49 ha with a total production of 2.01 million tons in Ethiopia (CSA 2018). In the country sesame seed yield is approximately 0.68 ton ha^{-1}, lower than a mean of 1 ton ha^{-1} reported in SSA (CSA 2018; FAOSTAT 2018). The low productivity is attributable to lack of improved varieties and modern production technologies. Also, abiotic stresses (recurrent drought and heat) and biotic factors (insect pests, diseases and weeds) are the major causes of low yields of the crop. The mean oil yield derived from locally cultivated varieties is 0.24 tons ha^{-1}. In Turkey, Iran and Pakistan, oil yields of 0.65, 0.57 and 0.29 ha^{-1} were reported, respectively (Caliskan et al. 2004; Tabatabaei et al. 2011; Jan et al. 2014;). Key yield influencing traits in sesame include number of branches per plant, number of capsules per plant, number of seeds per capsule, thousand seed weight, oil content and oil yield (Divya et al. 2018; Kalaiyarasi et al. 2019). Oil yield is the product of seed yield and oil content (Shimelis et al. 2008). Oil yield and quality are the major farmer and market-preferred traits of sesame

(Were et al. 2006; Dossa et al. 2018). This suggests that the next-generation sesame varieties should be high yielding with enhanced seed oil content.

Genetically diverse sesame genetic resources are collected and maintained by the Ethiopian Biodiversity Institute (EBI) (Woldesenbet et al. 2015). Farmers grow low yielding landracevarieties of sesame which have unique taste and aroma. Hence, the local sesame germplasm conserved by the EBI, landraces or farmersvarieties, and elite selections maintained by sesame research groupsprovide selection and breeding opportunities.

Genetic variation is key for successful selection and breeding gains for yield and yield components. The extent of phenotypic vari-ation in germplasm resources influences the expression of economic traits and the response to selection. Phenotypic variation of quantitative traits is attributable to the difference in the genetic con- stitution, environmental influence and genotype-by-environment interactions (Gadri et al. 2019). Quantitative traits are controlledby many genes each with minor genetic effects, and they are under the influence of genotype x environment interaction. Therefore, themagnitude of phenotypic variance (i.e. genotypic, environment and their interaction) influences the degree of trait heritability and re- sponse to selection. The magnitude and trend of association among key quantitative traits determines the success rate of simultaneous selection for diverse traits (Falconer & Mackay, 1996; Sleper & Poehlman, 2006). There are limited studies that examined the genetic variation, heritability, and association of yield-related traits in sesame using genetically diverse and representative populations. Previous studies reported the presence of greater variability forseed yield, oil content and yield-related traits (Gidey et al. 2012; Teklu et al. 2014; Hika et al. 2015; Iqbal et al. 2016).

Heritability measures the degree of resemblance between the offspring and their parents for a specific trait during selection. Heritability in the broad sense is the proportion of the genetic variation to the total phenotypic variation. It is the most useful genetic parameter in identically reproducing crops such as clonally propa- gated and self-fertilizing species. Narrow-sense heritability is the ratio of additive or heritable genetic variance to the total phenotypic variance. It is a useful selection parameter in cross-pollinated species and segregating breeding populations (Falconer & Mackay, 1996). High heritability estimates were reported in sesame for days-to-50% flowering, days-to-75% maturity, plant height, number of branches, number of capsules, number of seeds per capsule, thousand seed weight, seed yield per hectare and oil content in Ethiopia, India and Myanmar (Gidey et al. 2012; Hika et al. 2015; Teklu et al. 2014; Divya et al. 2018 Aye & Htwe, 2019).

Simple correlation and path coefficient analyses have been widely used to assessing associations of traits to guide selection of suitable genotypes possessing desirable economic traits (Abraha et al. 2016). Simple correlation analysis determines the linear relationship between two or more variables without pinpointing the cause and effect. Path coefficient analysis partitions the direct and indirect effect of one or more causal variable(s) upon a response variable. Path analysis helps to identify the most influential predictor variable(s) on a response variable (Dabholkar, 1992; Singh & Chaudhary, 1977). Path analysis studies in sesame reported that thousand seed weight had positive direct effect on seed yield indicating the importance of this trait for selection of high seed yielding genotypes (Gidey et al. 2012; Teklu et al. 2014; Aye & Htwe, 2019).

Knowledge on variance components and heritability are crucial in breeding programmes because it determines the inherited component of quantitative traits in future crossing and selection programmes. The heritability and expression of quantitative traits is dependent on the test populations and environments (Were et al. 2006; Wacal et al. 2019). Therefore, genetic characterisation and estimation of genetic parameters should involve diverse genetic pool under the target production environment. This will help to identify high-performing genotypes for breeding programmes aiming at seed yield, oil content and seed oil yield. The objective of this study was to determine the magnitude of phenotypic variance components, heritability and association of seed- and oil yield-related traits in diverse sesame collection to guide breeding.

3.2. Materials and Methods

3.2.1. Description of the study environments

The study was conducted at two locations, namely Humera (14°15' N latitude and 36°37' E longitude) and Kebabo (13°36' N latitude and 36°41' E longitude) in Ethiopia. The sites are situated in North West Ethiopia, a region known for its largest sesame production. The Humera and Kebabo sites are situated at the Humera Agricultural Research Center (HuARC) of the Ethiopian Institute of Agricultural Research (EIAR) and the Tigray Agricultural Research Institute (TARI), in that order. Humera and Kebabo are situated at an altitude of 609 and 696 meters and receive a total rainfall of 576.4 and 888.4 mm, respectively, during the main cropping season (June to August) in Ethiopia. The mean minimum and maximum temperatures at Humera site varied from 20.3 to 36.5°C. Kebabo has mean minimum and

maximum temperatures of 16.9 and 31.7°C. The predominant soil types of the sites are vertisol (Baraki et al. 2015).

3.2.2. Plant materials

The study used a collection of 100 diverse sesame genotypes and four released varieties (Table 3.1), called hereafter genotypes. The germplasm was originally collected from Amhara, Tigray, Afar, Oromia and Gambela regions in Ethiopia. The collections are maintained by the sesame and groundnut breeding programme of Werer Agricultural Research Centre of the EIAR. The test collections comprised of 95 accessions, one landrace (farmer variety) and four released varieties. The landrace variety ''Hirhir" is widely cultivated by farmers in the study areas. The four released varieties (i.e. 'Setit-1', 'Setit-2', 'Setit-3' and 'Humera-1') were developed by HuARC through mass selection among local germplasm collections.

Table 3.1. Descriptions of the 100 sesame genotypes used in the study.

Entry #	Collection name or designation	Source (regions or research center in Ethiopia)	Entry #	Collection name or designation	Source (regions in Ethiopia)
1	Hirhir Kebabo Hairless Sel-2	Tigray	32	ABXT-85-SEL-2-1	Afar
2	GXT=85(28-2)	Afar	33	Setit-2	21
3	Hirhir Kebabo Hairless-9	Tigray	34	Hirhir Kebabo Hairless-Sel-7	Tigray
4	NN-0068-1	Amhara	35	NN-0143	Amhara
5	NN-0108-2	Amhara	36	ACC NS-031	Oromia
6	NN-034	Amhara	37	NN-0029(2)	Amhara
7	BCS-0041	Afar	38	NN-0054	Amhara
8	ACC-031-5-14	Tigray	39	Morgo-Sel-P=13	Afar
9	NN-0129-2	Amhara	40	Hirhir Humera Sel-8	Tigray
10	ACC-203-020	Amhara	41	Tejareb Girar	Amhara
11	NN-0038-2	Amhara	42	NN0027	Amhara
12	Bawnji Fivel Kolet	Amhara	43	NN0009	Amhara
13	Gojam Azen(Aleka)	Amhara	44	ACC-203-610	Amhara
14	Hirhir Humera Sel-6	Tigray	45	NN-0146	Amhara
15	Bawnji Gobate	Amhara	46	NN-0044-2	Amhara
16	Shwarobit (83)	Amhara	47	NN-0018-2	Amhara
17	Humera-1	21	48	Hirhir Nigara 1st Sel-1	Tigray
18	ACC-202-950	Amhara	49	NN00136-1	Amhara
19	NN-0026	Amhara	50	NN-0088-2	Amhara
20	ACC-NO-041	Tigray	51	Hirhir Baeker-Sel-3	Tigray
21	ACC-203-612	Amhara	52	NN0068-3	Amhara
22	ACC-200-064-1	Tigray	53	NN0074-3	Amhara
23	Setit-1	21	54	NN0036-1	Amhara
24	Tejareb Kokit Sel-3	Amhara	55	Hirhir Kebabo Hairless Sel-4	Tigray
25	Orofalc ACC-2	Afar	56	NN0001-2	Amhara
26	NN-0022	Amhara	57	Bawnji Sel-2	Amhara
27	Setit-3	21	58	NN0058-2	Amhara
28	ABX=2-01-2	Afar	59	G-02	Amhara
29	NN-0020	Amhara	60	ACC-NS-007(2)	Oromia
30	ACC-NS-010	Oromia	61	GA-002(3)	Gambela
31	Hirhir Sel-2	Tigray	62	Endelemi Kirem Sel-2	Amhara

Table 3.1 (Continued).

Entry #	Collection name or designation	Source (Regions in Ethiopia)	Entry #	Collection name or designation	Source (Regions in Ethiopia)
63	ACC-205-299	Tigray	82	NN0061	Amhara
64	Hirhir Kebabo Early Sel-1	Tigray	83	ABXC-50402	Afar
65	NN0016-1	Amhara	84	NN0021	Amhara
66	Hirhir Adgeshu Sel -8	Tigray	85	NN0079-1	Amhara
67	NN0015	Amhara	86	ACC 202-333	Amhara
68	NN01-13	Amhara	87	NN-0052	Amhara
69	Bering Bawany	Afar	88	NN-0029-1	Amhara
70	Hirhir Nigara 1st Sel-2	Tigray	89	Teiahir Sanja Sel-6	Amhara
71	Gojam Azene (Yohans Sel-1)	Amhara	90	ACC-202-358	Amhara
72	NN0038-1	Amhara	91	NN0032	Amhara
73	NN0104	Amhara	92	NN0071	Amhara
74	Hirhir Kebabo Hairless Sel-6	Tigray	93	NN0064-1	Amhara
75	ACC 205-180	Tigray	94	NN0056	Amhara
76	Hirhir	Tigray	95	NN-01-03	Amhara
77	ACC 203-616	Amhara	96	NN0032-2	Amhara
78	NN0025	Amhara	97	Bawnji Maksegnt	Amhara
79	NN-0183-3	Amhara	98	Bawnji Flwha Sel-2	Amhara
80	Hirhir Humera	Tigray	99	NN0068-2	Amhara
81	NN0031	Amhara	100	Hirhir Filwha Large Seeded	Amhara

21= HuARC, Humera Agricultural Research Centre; and Tigray, Amhara, Afar, Oromia and Gambela are administrative regions in Ethiopia.

3.2.3. Experimental design and trial management

In each site, the experiment was laid out using a 10 x 10 simple lattice design with two replications. Each genotype was established in a plot size of 6.4m^2 consisting of 4 rows of 4m in length. The spacing between rows and plants was 40 and 10 cm, respectively. Fertilizer in the form of NPS applied at the rate of 100 kg/ha (19 kg N, 38 kg P_2O_5 and 7 kg S) and Urea was at the rate of 50 kg/ha (23 kg N). NPS and Urea were applied at the rates of 100 and 25 kg/ha at planting, while the remaining Urea (25 kg/ha) was applied between 30 and 40 days after emergence, following flower initiation based on the recommendation of HuARC (2010).

3.2.4. Data collection

The following quantitative data were collected based on whole plot basis from the two central rows: days-to-50% flowering (DF), days-to-75% maturity (DM), thousand seed weight (TSW, expressed in gram), seed yield per hectare (SYH) in t/ha, oil content (OC) in % and oil yield per hectare (OYH). DF was recorded as number of days from planting to the date when 50% of the plants showed flowers, while DM was recorded as number of days from planting to the date when 75% of the plants showed physiological maturity. TSW was measured from a random sample of 1,000 seeds of each genotype. SYH was measured in gram per plot and later converted into tonne (t) per hectare (ha). Oil content was determined using a FOSS NIR Systems model 5,000 near-infrared reflectance spectrometer (Foss NIR Systems Inc., Hillerod, Denmark). Oil yield per hectare was computed and expressed in tonne per hectare as the product of seed yield and percent oil content. Furthermore, the following data were collected from 10 randomly selected and tagged plants per collection at physiological maturity. Plant height (PH) was measured (in centimetres) from the base to the tip of the plant. Internode length (INL) was measured (in centimetres) between two consecutive nodes situated at the middle of the plant. Number of primary branches (NPB) that originated from the main stem of the plant was counted, number of secondary branches (NSB) that originated from the main branch of the plant was counted, number of capsules per plant was counted during 75% physiological maturity and number of seeds per capsule (NSPP) was counted using seed counter from a composite of three capsules per plant after harvest. Stem height from base to first branch (SHB) was measured as a distance from the base of the plant to first emerged primary branch during 75% maturity using a ruler and expressed in cm. The distance from base of lowest branch to first capsule (DFLBC) was measured as a distance between the lowest situated primary branch to first emerged capsule on the main stem during 75% maturity and expressed in cm. There was no insect pest damage during the study period.

3.2.5. Data analysis

3.2.5.1. Analysis of variance

Data collected were subjected to analysis of variance (ANOVA) using Proc lattice and Proc GLM procedures of SAS software version 9.4 (SAS Institute, 2018). A combined analysis of variance was performed using the following fixed model after Bartlett's homogeneity test of the error variances of individual location:

$$Y_{ijkl}=\mu + g_i + l_j + r_{k(j)} + b_{l(jk)} + e_{ijkl}$$

where Y_{ijkl}, represents the response variable, μ represents the overall mean, g_i represents the fixed effect of the genotype, l_j represents the fixed effect of the location, $r_{k(j)}$ represents the random effect of the replicate within the location, $b_{l(jk)}$ represents the random effect of the incomplete block within the location and the replicate, e_{ijkl} is the residual.

3.2.5.2. Estimates of variance components

Variance components for each trait were calculated from the combined analysis of variance and the expected mean squares (EMS) as presented in Table 3.2. Variance component was calculated using the EMS. These included the phenotypic coefficient of variance (PCV), genotypic coefficient of variance (GCV), and broad-sense heritability (h^2b). Genetic parameters were estimated according to Mather and Jinks (1971) using the following:

Table 3.2. Partial analysis of variance and expected mean squares for 100 sesame genotypes evaluated in two locations in Ethiopia.

Source of variation	df	Mean square	Expected mean squares
Genotype (g)	g-1	Ms_g	$\sigma^2_e + r\sigma^2_{gl} + l*r\sigma^2_g$
Location (l)	l-1	Ms_l	-
g x l	(g-1) (l-1)	Ms_{gl}	$\sigma^2_e + r\sigma^2_{gl}$
Error	l(g-1) (r-1)	Ms_e	σ^2_e
Total	gl-1		-

df degrees of freedom, σ^2_e environmental variance, σ^2_{gl} genotype-by-location interaction variance, σ^2_g genotypic variance, *r* number of replications, *l* number of locations Ms_g mean square values of genotypes for each source of variation of a trait

1. Phenotypic variance (σ^2p) =$\sigma^2g + \sigma^2gl /r + \sigma^2e /rl$
2. Genotypic variance (σ^2g) = $\frac{Mg- \sigma 2e}{rl}$

Where σ^2gl = Variance of genotype-by-location interaction, σ^2e = environmental variance, l = number of locations and r = number of replications, Mg=mean square value of genotypes

The broad-sense heritabilities (h^2b) were estimated as the ratio of the genotypic (σ^2g) to phenotypic variance (σ^2p) and expressed in percentage.

3.2.5.3. Correlation and path analysis

The magnitude of genetic correlations among traits was performed using R software version 4.0 (R Core Team, 2020). The student's t test was used to determine the level of significance of assessed traits at both 1% and 5% level of significance. Correlations were classified as (r = 0.2) weak, (r = 0.5) moderate and (r = 0.8) strong following Zou et al. (2003). Path analysis was conducted according to the procedure described by Dewey and Lu (1959). Seed yield and oil yield per hectare were considered as response variables. A path diagram was constructed to depict the direct and indirect effects of agro-morphological traits on seed and oil yield (Figures 3.1 and 3.2).

3.3. Results

3.3.1. Genotype, location and genotype- by- location interaction effects on agro- morphological traits and oil yield

The combined ANOVA also revealed significant ($p \leq 0.05$) genotype x location interaction for plant height, internode length, number of primary branches, number of secondary branches, distance from base of lowest branch to first capsule and seed yield per hectare (Table 3.3). The genotype effect was significant ($p \leq .05$) for days-to-50% flowering, days-to-75% maturity, plant height, internode length, number of secondary branches, number of seeds per capsule, distance from base of lowest branch to first capsule and seed yield per hectare.

Table 3.3. Analysis of variance showing mean square values and level of significance for the assessed agro-morphological characters, and oil yield of 100 sesame genotypes evaluated at two locations in Ethiopia.

Source of variation	Traits													
	d.f.	DF	DM	PH	INL	NPB	NSB	NCPP	NSPC	SHB	DFLBC	TSW	SYH	OYH
Rep (Loc)	2	171.27	205.65	714.76	0.00	121.72	28.62	14855.85	522.58	1730.39	374.90	17.61	0.00	0.06
Block (Loc*Rep)	36	5.05	16.04	240.28	1.16	1.15	0.14	276.21	249.16	70.61	111.98	6.36	0.05	0.07
Genotype (Gen)	99	6.93*	16.46*	360.33*	2.87**	0.61 ns	1.24**	145.00 ns	189.08**	70.52 ns	122.26**	5.43 ns	0.08**	0.07ns
Location (Loc)	1	201.64**	60.06*	423.33ns	852.35**	39.06**	35.40**	116.64ns	2787.84**	11306.07**	36898.56**	1.32ns	3.94**	1.47**
Gen x Loc	99	5.05 ns	14.03 ns	336.30*	3.43**	0.67*	0.68*	179.46ns	136.61 ns	48.88 ns	107.58**	5.49 ns	0.70**	0.05ns
Error	162	4.69	11.08	243.30	1.54	0.47	0.40	143.65	112.01	52.94	65.88	5.37	0.03	0.05

d.f. degrees of freedom, *DF* days-to-50 % flowering, *DM* days-to-75% maturity, *PH* plant height, *INL* inter node length, *NPB* number of primary branches per plant, *NSB* number of secondary branches per plant, *NCPP* number capsules per plant, *NSPC* number of seeds per capsule, *SHB* stem height from base to 1st branch, *DFLBC* distance from base of lowest branch to 1st capsule, *TSW* thousand seeds weight, *SYH* seed yield per hectare, *OYH* oil yield per hectare.

Gen x Env genotype by environment interaction, * and ** denote significance difference at 5% and 1% levels of probability, respectively; *ns* non-significant.

3.3.2. Genotypic and phenotypic coefficients of variation, and broad-sense heritability of seed yield and related-traits and seed oil yield

Genotypic (σ^2_g), phenotypic (σ^2p) and genotype by location interaction (σ^2gl) variance components, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV) and broad-sense heritability (h^2b) for studied traits among 100 sesame genotypes are presented in Table 3.4. GCV values ranged from 1.26% for days-to-75% maturity to 29.46% for oil yield per hectare. The highest GCV values of 29.46% and 23.29% were recorded for oil yield per hectare and seed yield per hectare, respectively. PCV values ranged from 3.14% for days-to-75% maturity to 72.17% for oil yield per hectare. Likewise, the highest PCV values of 72.17% and 57.29% were recorded for oil yield per hectare and thousand seed weight, in that order. Broad-sense heritability values ranged from 2.07% for number of capsules per plant to 38.18% for number of secondary branches. High heritability estimates of 38.18% and 30.73% were recorded for number of secondary branches and days-to-50% flowering. Similarly, medium heritability estimates of 26.32% and 20.76% were recorded for seed yield per hectare and distance from base of lowest branch to first capsule, in that order.

Table 3.4. Traits variance components [phenotypic variance (σ^2_p), genotypic variance (σ^2_g), genotype by location interaction variance (σ^2_{gl})], mean values, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), and broad-sense heritability (h^2b) among 100 sesame genotypes evaluated across two locations in Ethiopia

Traits	σ^2_g	σ^2_{gl}	σ^2_p	Mean	H^2b (%)	GCV (%)	PCV (%)
DF	0.56	2.71	3.09	43	30.73	1.74	3.14
DM	1.35	8.49	8.36	92	16.09	1.26	3.14
PH (cm)	29.26	214.65	197.41	119.6	14.82	4.52	11.75
INL (cm)	0.33	2.66	2.05	9.2	16.24	6.27	15.55
NPB	0.04	0.44	0.37	4	9.46	4.68	15.21
NSB	0.21	0.48	0.55	2	38.18	22.91	37.08
NCPP	1.48	67.85	71.31	41	2.07	2.96	20.60
NSPC	8.58	124.13	98.31	51	8.73	5.74	19.44
SHB (cm)	4.40	22.41	28.84	24.4	15.24	8.59	22.01
DFLBC (cm)	14.10	74.64	67.89	39.8	20.76	9.43	20.70
TSW (gm)	0.01	2.81	2.76	2.9	0.54	4.22	57.29
SYH (ton ha^{-1})	0.01	0.06	0.05	4.8	26.32	23.29	45.41
OYH (ton ha^{-1})	0.01	0.03	0.03	2.5	16.67	29.46	72.17

DF days-to-50 % flowering, *DM* days-to-75% maturity, *PH* plant height, *INL* inter node length, *NPB* number of primary branches per plant, *NSB* number of secondary branches per plant, *NCPP* number capsules per plant, *NSPC* number of seeds per capsule, *SHB* stem height from base to 1st branch, *DFLBC* distance from base of lowest branch to 1st capsule, *TSW* thousand seeds weight, *SYH* seed yield per hectare, *OYH* oil yield per hectare.

3.3.3 Correlations of seed yield and yield-related traits and seed oil content among sesame genotypes

Genetic correlation coefficients for assessed traits among 100 sesame genotypes is presented in Table 3.5. Positive and moderately high correlation was computed for seed yield with oil yield ($r = 0.57$; $p < 0.01$), whereas low and positive correlations were recorded between seed yield with number of capsules per plant ($r = 0.29$; $p < 0.01$), number of seeds per capsule ($r = 0.42$; $p < .01$), thousand seed weight ($r = 0.31$; $p < .01$) and oil content ($r = 0.25$; $p < .01$). Low and negative correlation was recorded between seed yield and days-to-75% maturity ($r = -0.24$; $p < .05$), and between days-to-50% flowering and thousand seed weight ($r = -0.25$; $p < 0.05$).

3.3.4. Path coefficient analysis for assessed traits of sesame genotypes

Direct and indirect effects of the studied agro-morphological traits on seed oil content (Figure 3.1 and Table 3.6) and seed yield (Figure 3.2 and Table 3.7) are presented. Oil yield and number of seeds per capsule had high direct path values of 0.42 and 0.30 on seed yield per hectare, respectively. Thousand seed weight and oil content also recorded moderate and significant direct effect value of each 0.18 on seed yield per hectare (Table 3.6). Number of capsules per plant and thousand seed weight recorded moderate and significant direct effect values of 0.17 and 0.09 on oil yield per hectare, respectively (Table 3.7). The highest direct effect value of 0.57 was observed between seed yield per hectare and oil yield per hectare.

Table 3.5. Genotypic correlations coefficients for agro-morphological traits, and seed oil content oil yield of 100 sesame genotypes tested across two locations in Ethiopia.

Traits	DM	PH	INL	NPB	NSB	NCPP	NSPC	SHB	DFLBC	TSW	OC	OYH	SYH
DF	0.59**	-0.02ns	-0.12ns	-0.34**	0.12ns	0.21*	-0.00ns	-0.04ns	0.20*	-0.25*	-0.08ns	-0.09ns	-0.07ns
DM	1.00	0.03ns	-0.00ns	-0.43**	0.14ns	0.02ns	-0.07ns	0.12ns	0.29**	-0.10ns	-0.08ns	-0.07ns	-0.24*
PH		1.00	0.03ns	-0.06ns	0.03ns	-0.01ns	0.22*	0.09ns	0.17ns	0.08ns	-0.00ns	0.10ns	0.10ns
INL			1.00	0.01ns	0.05ns	0.00ns	0.09ns	0.14ns	0.09ns	0.11ns	0.00ns	0.02ns	0.16ns
NPB				1.00	0.08ns	-0.17ns	-0.11ns	0.21*	-0.21*	-0.10ns	0.05ns	-0.10ns	0.08ns
NSB					1.00	0.07ns	0.22*	0.15ns	-0.00ns	0.02ns	0.09ns	0.16ns	0.18ns
NCPP						1.00	0.34**	0.01ns	0.13ns	-0.08ns	-0.03ns	0.32**	0.29**
NSPC							1.00	0.08ns	0.23*	0.03ns	-0.11ns	0.33**	0.42**
SHB								1.00	0.23*	-0.02ns	0.02ns	0.06ns	-0.06ns
DFLBC									1.00	-0.10ns	-0.18ns	0.18ns	-0.09ns
TSW										1.00	0.18ns	0.27**	0.31**
OC											1.00	0.11ns	0.25*
OYH												1.00	0.57**

DF days-to-50 % flowering, *DM* days-to-75% maturity, *PH* plant height, *INL* inter node length, *NPB* number of primary branches per plant, *NSB* number of secondary branches per plant, *NCPP* number capsules per plant, *NSPC* number of seeds per capsule, *SHB* stem height from base to 1st branch, *DFLBC* distance from base of lowest branch to 1st capsule, *TSW* thousand seeds weight, *SYH* seed yield per hectare, *OYH* oil yield per hectare.

* and ** denote significance difference at 5% and 1% levels of probability, respectively; *ns* non-significant

Table 3.6. Direct (diagonal and boldfaced scripts) and indirect (off-diagonal) path coefficients of assessed traits with seed yield per hectare as a response variate among 100 sesame collections tested across two locations in Ethiopia.

Traits	DF	DM	PH	INL	NPB	NSB	NCPP	NSPC	SHB	DFLBC	TSW	OC	OYH	SYHrg
DF	**0.20**	-0.08	-0.00	-0.01	-0.06	0.00	0.02	-0.00	0.00	-0.03	-0.04	-0.01	-0.04	-0.07ns
DM	0.11	**-0.14**	0.00	-0.00	-0.08	0.00	0.00	-0.02	-0.01	-0.04	-0.01	-0.01	-0.03	-0.24*
PH	-0.00	-0.00	**0.04**	0.00	-0.01	0.00	-0.00	0.06	-0.01	-0.02	0.01	-0.00	0.04	0.10ns
INL	-0.02	0.00	0.00	**0.15**	0.00	0.00	0.00	0.02	-0.01	-0.01	0.02	0.00	0.01	0.16ns
NPB	-0.06	0.06	-0.00	0.00	**0.19**	0.00	-0.01	-0.03	-0.02	0.03	-0.01	0.00	-0.04	0.08ns
NSB	0.02	-0.02	0.00	0.00	0.01	**0.01**	0.00	0.06	-0.01	0.00	0.00	0.01	0.07	0.18ns
NCPP	0.04	-0.00	-0.00	0.00	-0.03	0.00	**0.09**	0.10	-0.00	-0.02	-0.01	-0.00	0.14	0.29**
NSPC	-0.00	0.01	0.00	0.01	-0.02	0.00	0.03	**0.30**	-0.01	-0.03	0.00	-0.02	0.14	0.42**
SHB	-0.00	-0.01	0.00	0.02	0.04	0.00	0.00	0.02	**-0.12**	-0.03	-0.00	0.00	0.02	-0.06ns
DFLBC	0.04	-0.04	0.00	0.01	-0.04	0.00	0.01	0.07	-0.02	**-0.16**	-0.01	-0.03	0.07	-0.09ns
TSW	-0.05	0.01	0.00	0.01	-0.01	0.00	-0.00	0.00	0.00	0.01	**0.18**	0.03	0.11	0.31**
OC	-0.01	0.01	0.00	0.00	0.01	0.00	-0.00	-0.03	-0.00	0.02	0.03	**0.18**	0.05	0.25*
OYH	-0.01	0.01	0.00	0.00	-0.02	0.00	0.03	0.10	-0.00	-0.02	0.05	0.02	**0.42**	0.57**

Residual effect= 0.421, *DF* days-to-50 % flowering, *DM* days-to-75% maturity, *PH* plant height, *INL* inter node length, *NPB* number of primary branches per plant, *NSB* number of secondary branches per plant, *NCPP* number capsules per plant, *NSPC* number of seeds per capsule, *SHB* stem height from base to 1st branch, *DFLBC* distance from base of lowest branch to 1st capsule, *TSW* thousand seeds weight, *SYH* seed yield per hectare, *OYH* oil yield per hectare.

* and ** denote significance difference at 5% and 1% level of probability, respectively; *ns* non-significant.

Table 3.7. Direct (diagonal and boldfaced scripts) and indirect (off-diagonal) path coefficients of assessed traits with oil yield per hectare as a response variate among 100 sesame collections tested across two locations in Ethiopia.

Traits	DF	DM	PH	INL	NPB	NSB	NCPP	NSPC	SHB	DFLBC	TSW	OC	SYH	OYHrg
DF	**-0.21**	0.02	0.00	0.02	0.05	0.01	0.04	0.00	0.00	0.05	-0.02	0.00	-0.04	-0.09ns
DM	-0.12	**0.03**	0.00	0.00	0.06	0.01	0.00	0.00	0.01	0.07	-0.01	0.00	-0.14	-0.07ns
PH	0.01	0.00	**-0.01**	0.00	0.01	0.00	0.00	-0.01	0.01	0.04	0.01	0.00	0.06	0.10ns
INL	0.02	0.00	0.00	**-0.13**	0.00	0.01	0.00	0.00	0.01	0.02	0.01	0.00	0.09	0.02ns
NPB	0.07	-0.01	0.00	0.00	**-0.15**	0.01	-0.03	0.00	0.02	-0.05	-0.01	0.00	0.05	-0.10ns
NSB	-0.02	0.00	0.00	-0.01	-0.01	**0.09**	0.01	-0.01	0.01	0.00	0.00	0.00	0.10	0.16ns
NCPP	-0.04	0.00	0.00	0.00	0.03	0.01	**0.17**	-0.01	0.00	0.03	-0.01	0.00	0.16	0.32**
NSPC	0.00	0.00	0.00	-0.01	0.02	0.02	0.06	**-0.04**	0.01	0.05	0.00	0.00	0.23	0.33**
SHB	0.01	0.00	0.00	-0.02	-0.03	0.01	0.00	0.00	**0.07**	0.05	0.00	0.00	-0.03	0.06ns
DFLBC	-0.04	0.01	0.00	-0.01	0.03	0.00	0.02	-0.01	0.02	**0.23**	-0.01	0.00	-0.05	0.18ns
TSW	0.05	0.00	0.00	-0.01	0.02	0.00	-0.01	0.00	0.00	-0.02	**0.09**	0.00	0.17	0.27**
OC	0.02	0.00	0.00	0.00	-0.01	0.01	-0.01	0.00	0.00	-0.04	0.02	**-0.01**	0.14	0.11ns
SYH	0.02	-0.01	0.00	-0.02	-0.01	0.02	0.05	-0.02	0.00	-0.02	0.03	0.00	**0.55**	0.57**

Residual effect= 0.394, *DF* days-to-50 % flowering, *DM* days-to-75% maturity, *PH* plant height, *INL* inter node length, *NPB* number of primary branches per plant, *NSB* number of secondary branches per plant, *NCPP* number capsules per plant, *NSPC* number of seeds per capsule, *SHB* stem height from base to 1st branch, *DFLBC* distance from base of lowest branch to 1st capsule, *TSW* thousand seeds weight, *SYH* seed yield per hectare, *OYH* oil yield per hectare.

* and ** denote significance difference at 5% and 1% level of probability, respectively; *ns* non-significant.

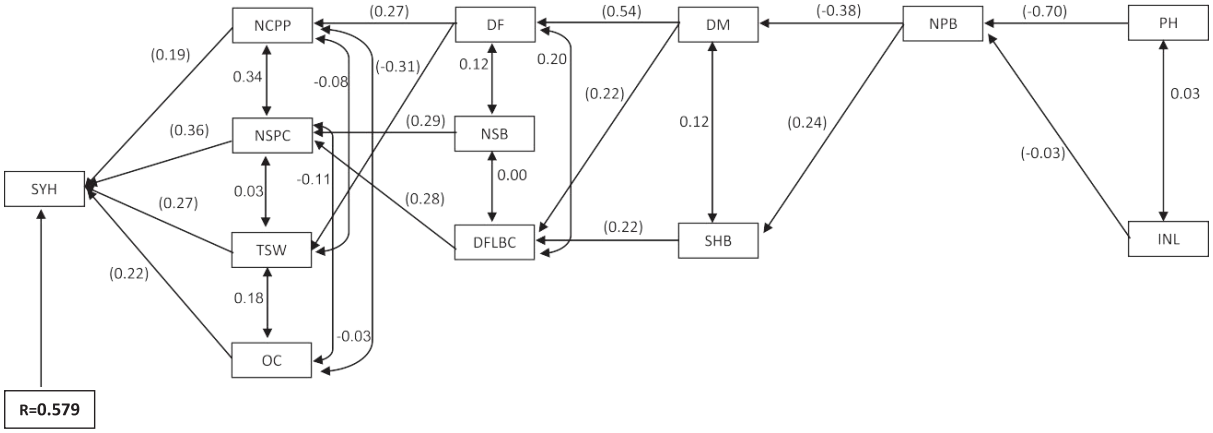

Figure 3.1. Path diagram displaying causal relationships of assessed agro-morphological traits on the response variable (seed yield) among 100 sesame genotypes tested across two locations in Ethiopia. Values in parenthesis are direct path coefficients while other values are correlation coefficients. DF, days-to-50% flowering; DFLBC, distance from base of lowest branch to 1st capsule; DM, days- to-75% maturity; INL, internode length; NCPP, number capsules per plant; NPB, number of primary branches per plant; NSB, number of secondary branches per plant; NSPC, number of seeds per capsule; PH, plant height, SHB, stem height from base to 1st branch; SYH seed yield per hectare; TSW, thousand seed weight; R, residual effects.

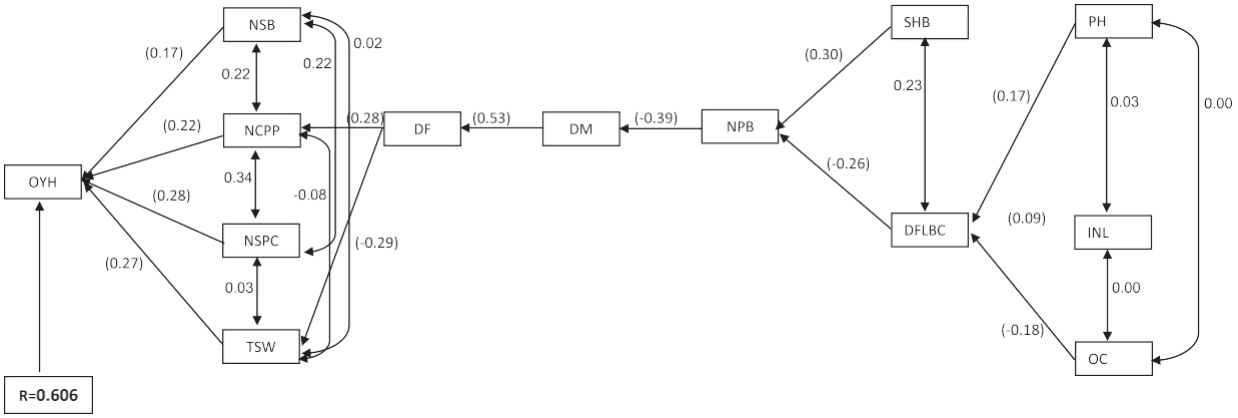

Figure 3.2. Path diagram displaying causal relationships of assessed agro-morphological traits on oil yield among 100 sesame genotypes tested across two locations in Ethiopia. Values in parenthesis are direct path coefficients while other values are correlation coefficients. DF, days-to-50% flowering; DFLBC, distance from base of lowest branch to 1st capsule; DM, days-to-75% maturity; INL, internode length; NCPP, number capsules per plant; NPB, number of primary branches per plant; NSB, number of secondary branches per plant; NSPC, number of seeds per capsule; OYH, oil yield per hectare; PH, plant height, SHB, stem height from base to 1st branch; TSW, thousand seed weight; R, residual effects.

3.4. Discussion

Sesame is an important seed oil crop globally. It is the leading oil crop in Ethiopia in terms of area coverage and total production serving for local and international markets. Understanding the underlying genetic mechanisms influencing seed and oil yields is important for developing improved sesame genotypes possessing desirable agronomic traits, oil quality and quantity. This will enable selection of high-performing cultivars for direct production, industrial use and breeding programmes.

The current study examined variance components and heritability of seed yield, yield-related traits, and seed oil content among 100 genetically diverse sesame genotypes. The study recorded high phenotypic and genotypic variations for the assessed traits, indicating the effectiveness of selection (Table 3.4). According to Deshmukh et al. (1986), phenotypic and genotypic coefficient of variation values greater than 20% are regarded as high, whereas values between 10% and 20% are medium and values less than 10% regarded as low. In this study, the oil yield per hectare, seed yield per hectare and number of secondary branches recorded high phenotypic and genotypic coefficients of variation ≥20%. This is closer to values reported by several studies (Gidey et al. 2012; Hika et al. 2015; Teklu et al. 2014; Iqbal et al. 2016). The observed phenotypic variability among the sesame genetic resource of Ethiopia indicated the correspondence between the genotypic and phenotypic variances, and the possible high response to selection in the future breeding programmes.

Heritability is of interest in crop improvement programmes to measure the value of selection for targeted economic traits. Understanding the heritability of agronomic and nutritional traits is vital to improve selection response for sesame breeding and cultivar development programmes. According to Robinson (1966), heritability values from 30% to 60% are considered high, whereas values ranging between 10% and 30% medium and values from 5% to 10% are regarded as low. In the present study, heritability in broad sense values was higher for the number of secondary branches and days-to-50% flowering (Table 3.4), indicating high correspondence between the genotypic and phenotypic variances, and hence higher response to selection. Hika et al. (2015) reported very high heritability (>80%) for DF, DM, PH, NPB, NSB, NCPP and SYH when assessing 64 sesame germplasm collections from Ethiopia. However, Teklu et al. (2014) reported moderate heritability (40 to 59%) for PH, INL and NSPC in another 64 sesame populations collected from the Amhara and Tigray regions in Ethiopia.

High heritability values provide a measure of genetic advancement through phenotypic selection (Johnson et al. 1955). In the present study, higher heritability was computed for days-to-50 %

flowering and number of secondary branches. Conversely, Divya et al. (2018) and Aye and Htwe (2019) reported high heritability and high genetic advance values for plant height and number of capsules per plant in Indian and Myanmar sesame germplasm collections. Traits with high heritability and high genetic advance are reported to be under the control of additive gene action. Hence, phenotypic selection can be an effective approach (Panse, 1957). In the present study, sesame genotypes, including Hirhir Kebabo Hairless-9, 'Setit-3', Orofalc ACC-2, Hirhir Humera Sel-6, 'Setit-1' and ACC-NS-007(2), were identified with high seed and oil yields. The identified genotypes are useful for production in targeted environments in Ethiopia and/or as parental lines to develop sesame populations for future selection and genetic advancement.

Association analysis between economic traits and their components is vital to improve selection efficiency for breeding. In the present study, genetic correlation analysis revealed positive and highly significant associations between seed yield per hectare with thousand seed weight, and oil yield per hectare and number of seeds per capsule and number of capsules per plant with oil yield (Table 3.5). Hika et al. (2014) and Aye and Htwe (2019) reported positive and highly significant associations of seed yield with plant height, number of primary branches and number of capsules per plant. Correlation among traits is attributable to linked genes or pleiotropic genetic effects causing the traits to change in the same direction (Falconer & Mackay, 1996). Selection based on highly correlated traits, mainly seed and oil yield ($r = 0.57$; $p < .001$), would lead to improvement of both traits among the studied sesame genotypes.

Path coefficient analysis allows identification of secondary traits with direct and indirect effects on the response traits to aid selection. The present study revealed that oil yield and number of seeds per capsule can be directly selected to improve seed yield per hectare. Number of seeds per capsule positively influenced seed yield through oil yield (Figure 3.1 and Table 3.6). This suggests that genotypes with high oil yield were more likely to have higher number of seeds per capsule. The residual value (0.421) indicates that characters which are included in the genotypic path analysis explained 57.9% of the total variation in seed yield which indicates that there may be some more components that are contributing towards seed yield. Similarly, seed yield and thousand seed weight can directly be selected to improve oil yield per hectare. The seed yield had indirect effect on oil yield through number of capsules per plant, suggesting that genotypes which have higher number of capsules per plant were more likely to have higher seed yield (Figure 3.2 and Table 3.7). The residual (0.394) indicates that characters which are included in the genotypic path analysis explained 60.6% of the total variation in oil yield which indicates that there may be some more components that are

contributing towards oil yield. Therefore, more components should be included in future studies to explain more total variation to wards oil yield improvement. Unlike the present findings, Hika et al. (2014) reported positive and high direct effects of days-to-50% flowering and harvesting index on seed yield.

3.5. Conclusions

The current study determined genotypic and phenotypic variation, heritability and genetic advance of seed yield and yield-related traits, seed oil yield and oil content in genetically differentiated and unique sesame germplasm collections of Ethiopia. The tested sesame genotypes showed a wide range of variation for seed and oil yields and related traits. The following sesame genotypes, namely Hirhir Kebabo Hairless-9, 'Setit-3', Orofalc ACC-2, Hirhir Humera Sel-6, 'Setit-1' and ACC-NS-007(2) with high seed and oil yields, and oil content were identified. These genotypes would possibly be useful as breeding parents for improving genetic gains in sesame with enhanced seed and oil yields and oil quality and should be promoted for further field stability evaluation, which might be utilized in future sesame breeding programmes and production.

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Chapter 4. Genetic diversity and population structure of Ethiopian sesame (*Sesamum indicum* L.) germplasm assessed through phenotypic traits and simple sequence repeat markers

Abstract

Ethiopia is one of the centers of genetic diversity of sesame (*Sesamum indicum* L.). The sesame genetic resources present in the country should be explored for local, regional, and international genetic improvement programs to design high-performing and market-preferred varieties. This study's objective was to determine the extent of genetic diversity among 100 cultivated sesame germplasm collections of Ethiopia using phenotypic traits and simple sequence repeat (SSR) markers to select distinct and complementary genotypes for breeding. One hundred sesame entries were field evaluated at two locations in Ethiopia for agro-morphological traits and seed oil content using a 10 × 10 lattice design with two replications. The test genotypes were profiled using 27 polymorphic SSR markers at the Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences. Analysis of variance revealed significant ($p \leq 0.05$) entry by environment interaction for plant height, internode length, number of secondary branches, and grain yield. Genotypes such as Hirhir Kebabo Hairless-9, Setit-3, Orofalc ACC-2, Hirhir Humera Sel-6, ABX = 2-01-2, and Setit-1 recorded grainyield of >0.73 ton ha^{-1} with excellent performance in yield component such as oil yield per hectare. Grain yield had positive and significant ($p < 0.01$) associations with oil yield (r = 0.99), useful for simultaneous selection for yield improvement in sesame. The SSR markers revealed gene diversity and polymorphic information content values of 0.30 and 0.25, respectively, showing that the tested sesame accessions were genetically diverse. Cluster analysis resolved the accessions into two groups, while population structure analysis revealed four major cluster groups, thus enabling selection and subsequent crossing to develop breeding populations for cultivar development. Based on phenotypic and genomic divergence, the following superior and complementary genotypes were selected for use in future sesame breeding programs: Hirhir Humera Sel-6, Setit-3, Hirhir Kebabo Hairless Sel-4, Hirhir Nigara 1st Sel-1, Humera-1 and Hirhir KebaboEarly Sel-1 (from cluster II-a), Hirhir kebabo hairless-9, NN-0029(2), NN0068-2 and Bawnji Fiyel Kolet, (from cluster II-b). The selected genotypes will serve as parents in the local breeding program in Ethiopia.

Keywords: agronomic traits; Ethiopia; genetic diversity; microsatellites; population structure; principal component analysis; *Sesamum indicum*

4.1. Introduction

Sesame (*Sesamum indicum* L.) is a multi-purpose high-value oilseed crop. It is a global commodity serving the food, feed, and cosmetic industries. The seed oil content of sesame is about 60%, the highest when compared with other oilseed crops such as sunflower (~45%), rapeseed (~40%), and soybean (~20%) [1–4]. Sesame oil comprises about 85% unsaturated and 15% saturated fatty acids. The fatty acid contains linoleic acid (~46%), oleic acid (~38%), palmitic acid (~12%), and stearic acid (~4%) [4–7]. Sesame seed is a rich source of protein (≈24%), carbohydrate (≈13.5%), vitamins (e.g., A and E), lignans (sesamin and sesamolin), γtocopherol, phytosterols (β-sitosterol and Campesterol), policosanols (Docosanol, Tetracosanol, Hexacosanol, and Octacosanol) and lipids [4,7–9]. These attributes make sesame a 'superfood' comprising all the essential human nutrients in desirable proportions.

Sesame is the second most valuable export crop after coffee (*Coffea arabica* L.) and a major contributor to Ethiopia's gross domestic product [10]. In Ethiopia, the area allocated to sesame production in 2018 was 294,819.49 ha, approximately 39.4% of the total estimated area allocated for oil crops production [11]. Compared with global sesame production, Ethiopia ranks eight with a total annual production of 301,302 tons after Sudan (981,000 tons), Myanmar (768,858 tons), India (746,000 tons), Nigeria (572,761 tons), Tanzania (561,103 tons), China (433,386 tons), and China Mainland (431,500 tons) [12].

Ethiopia is the center of origin and diversity for the cultivated sesame and its allied species. The Ethiopian Biodiversity Institute (EBI) maintains one of the most extensive collections of sesame genetic resources in Africa. About 5000 genetically diverse sesame germplasm resources are conserved by the EBI [13]. The germplasm pool can provide various unique economic traits and gene combinations for global sesame improvement. However, the genetic resources maintained at the EBI are yet to be explored for local, regional, and international sesame improvement programs to develop high-performing and market- preferred varieties. Ethiopia's mean sesame yield is 0.68 tons ha−1, which is relatively low compared with a mean yield of 1 ton ha−1 in sub-Saharan Africa and 1.29 tons ha−1 in Egypt [11,12]. The low productivity is attributable to lack of improved and high-yielding varieties and traditional production technologies, among other constraints. Landrace varieties are the main sources of seed for cultivating the crop in Ethiopia. Landraces are inherently low yielders and prone to capsule shattering leading to reduced productivity. However, landraces are highly valued for possessing intrinsic farmer-preferred attributes such as unique taste and aroma, and adaptation to marginal growing conditions that often characterize low input farming

systems [8,14].

Sesame genetic resources maintained at the EBI can be explored to search for new sources of useful genetic variation for economic traits. This includes grain yield and yield- components, resistance to diseases and insect pests, tolerance to abiotic stresses, capsule shattering tolerance, and nutritional quality. This will identify desirable and complementary parents useful for gene discovery. Hence, rigorous phenotyping and genotyping can establish genetic polymorphism in the germplasm pool and classify the genotypes into distinct clusters for ideotype breeding.

Previous studies have reported considerable phenotypic diversity for agronomic and quality traits in Ethiopia's sesame genetic resources [15–18]. However, these studies did not fully represent the landrace collections from various parts of Ethiopia. Hence there is a need for a comprehensive assessment of the genetic diversity present in the Ethiopian sesame using a relatively more significant number of genotypes representing the diverse germplasm resources and sampled from various regions through phenotypic traits and effective molecular markers.

Several molecular markers such as amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), microsatellites or simple sequence repeat (SSR), and single nucleotide polymorphism (SNP) markers are widely used in genetic diversity analysis of various crop genetic resources. SSR or microsatellites have been commonly used in genetic diversity studies on sesame [19–22]. The SSRs are preferred for their ability to detect high degrees of polymorphism, higher reproducibility, and abundant coverage of the genome [20,21]. Moreover, SSR markers can be used for loci with multiple co-dominant alleles [23]. Wei et al. [20] and Asekova et al. [21] assessed genetic diversity and population structure present in sesame genetic resources sampled from China and Korea using 44 and 23 SSRs, respectively. The authors reported two and three major clusters among the Chinese and Korean collections, respectively. The level of genetic diversity varies among different germplasm populations and environmental conditions, suggesting that each set of populations must be assessed in a target production environment for selection and genetic grouping. Therefore, this study's objectives were to determine the extent of genetic diversity among 100 diverse sesame germplasm collections of Ethiopia using phenotypic traits and simple sequence repeat markers to select and recommend distinct and complementary parents for direct production, breeding, and conservation.

4.2. Materials and Methods

4.2.1. Plant materials

The study used a collection of 100 sesame entries originally collected from the Amhara, Tigray, Afar, Oromia, and Gambela regions in Ethiopia. The details of the germplasm collections used in the study are presented in Section 3.2.2.

4.2.2. Phenotyping

4.2.2.1. Description of the Study Sites

The study was conducted in northwestern Ethiopia at two selected locations, namely, Humera (14◦15′ N, 36°37′ E) and Kebabo (13°36′ N, 36◦41′ E). The descriptions of the study environments are summarised in Section 3.2.1.

4.2.2.2. Experimental Design and Trial Management

The experiment was conducted under field conditions and laid out using a 10 X 10 simple lattice design, with two replications, at each site as outlined in Section 3.2.3.

4.2.2.3. Phenotypic Data Collection

Data were collected on quantitative and qualitative traits as described in Section 3.2.4.

4.2.2.4. Phenotypic Data Analysis

The phenotypic data were subjected to analysis of variance (ANOVA) using the alpha-lattice and general linear model (GLM) procedures of the SAS software version 9.4 [24]. A combined analysis of variance across the two locations was performed after Bartlett's homogeneity test of variance. Mean comparisons among accessions were performed using Tukey's Honestly Significant Difference (HSD) test procedure at 5% level of significance used to identify significant differences among genotype means. The correlation among traits was performed using R software version 4.0 [25] to determine the magnitude of associations among the studied traits. Multivariate analysis using the principal components was performed using R software version 4.0 [25].

4.2.3. Genotyping

4.2.3.1. DNA Extraction, Primer Selection, Polymerase Chain Reaction, and Electrophoresis

The 100 sesame entries were planted at the Oil Crops Research Institute (OCRI)—the Chinese Academy of Agricultural Sciences (OCRI-CAAS), China. Ten seeds per entry were sown in a plastic tray in a growth room. Three two-weeks old seedlings were randomly selected from each entry, and fresh young leaves were collected and ground in liquid nitrogen for DNA extraction. The DNA was extracted following the Cetyl-tetramethyl ammonium bromide (CTAB) method. Approximately 200 mg of ground plant tissue combined with 500 µL of CTAB buffer was incubated in a water bath at 65 ^{O}C, 4 times for 10 min, and subjected to centrifugation (Eppendorf) at 12,000 rpm for 10 min at 4 ^{O}C. The supernatant was then transferred into new 5 mL micro-tubes, and 400 µL chloroform: iso-amyl alcohol (24:1) was added into the tubes and mixed gently. After a minute of centrifugation (Eppendorf) (centrifuged at 12,000 rpm for 10 min at 4 ^{O}C), the supernatant was transferred into new 5 mL micro-tubes, and 400 µL isopropanol was added into the tubes, mixed gently and kept at 20 ^{O}C for 30 min and subjected to centrifugation at 12,000 rpm for 10 min at 4 ^{O}C. The precipitated DNA was washed by 75% ethanol three times. The resulting pellet was dried under vacuum and dissolved in 100 uL DD H2O. DNA concentrations were measured using the Quantus TM Fluorometer (Promega Corporation, Madison, USA). Microsatellites from 13 linkage groups were designed and used for the following experiments. Twenty-seven primers were selected because of their suitability in discriminating sesame genotypes. The presently used primers were initially selected amongst 160 candidate primers based on their higher polymorphic information content and provided clear and informative amplicon profiles in sesame genetic analysis [26].

The polymerase chain reaction (PCR) conditions were maintained as follows; each PCR reaction was carried out in a 20 µL solution containing 25 ng of DNA, 4 µmol of forward primers, 4 µmol of reverse primers, 1 buffer, 0.25 mmol of dNTPs, and 0.80 U Taq polymerase. The temperature profile used for PCR amplification comprised a denaturation step at 94 ^{O}C for 1 min, followed by primer annealing temperature at 45.2–53 ^{O}C for 1 min, and elongation at ^{O}C for 1 min. After 34 cycles, the reaction was terminated with a 10 min final extension time at 72 ^{O}C.

The PCR reaction conditions were the same for all the primers, except for the annealing temperatures. The PCR products were electrophoresed on 6% Acrylamide gel (=200 mL of 5 T.B.E., 420 g of Urea [H2NCONH2], 75 g of Acrylamide, 3 g of Bis-Acrylamide, and 400 mL of distilled water) at a voltage

value of 2000, current 300 A, power 80 W for 1:30 h. After silver staining, the bands on the gels were recorded, and a total of 27 markers (Table 4.1) with high polymorphism were used for capillary electrophoresis. The PCR products were separated by capillary electrophoresis on an ABI 3730 automatic sequencer. The marker data was presented as fragment sizes in an excel spreadsheet.

Table 4.1. Description of the SSR markers used in the study.

No	Primers	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	Product size (bp)
1	ID0046	TCAACGTGATTGCTCCCATA	CAGCTGCCTGAAAGAAGAGG	101
2	ZMM1043	CCCGAAAATAGGATTTCTAACCA	TTTTGGACTGCTATTGAGGGA	184
3	ZMM3261	CGAAAGCATGAGACGAGTATG	AACTAGTGCGCAATTCATTCAA	244
4	ID0041	AGGCTTTCACATCATCAAATG	CATGTAGGATGCAACTCTTCAAA	280
5	ZMM5015	ATTTATTGGGTTGCTGGGAA	TGAAAATTAAGTCACCAGTACCACC	151
6	ZMM4664	CCTTCACTTCAAATCCGTCAA	TTTGGTTTGCATAGATGCTCTT	184
7	ZMM1809	TTAAGCCTCGTTGACTCCAA	ATTGTACGGCATGTTGTCCC	256
8	ZMM2321	CAACACCACCAACGCATATC	AGCAACGATTCACGACATTG	280
9	ZMM5358	TAGGATGCTTTGAATTGGGC	AGGAACAAACATACGGCGTC	164
10	ID0068	TCTTCGGAGTTAACACCCTCA	TTGGATTTCCATGTATGCCA	199
11	ZMM3312	GCAAAATCTTCTTTCCTCCG	GCAGCAAGGGAATTGAATGT	264
12	ZMM1033	CGTAGTGGTTCCCCTCACAT	ATGCTTTCCCCCAAATAACC	179
13	ZMM1189	TATCCAGGGGAAAACCAGAA	TTGGATTTTCCTTCTCACGC	212
14	ZMM2202	TCAGGAAGAAAGAATTGCTGC	CAATTTAACCATCCTGACTC	276
15	ZMM1637	GCGGTGACATATTAAGGGCA	ACCGGAATCCGAACATGTAA	265
16	ZMM4645	TTGAGCGATTCATCGACTTG	TTCTCCGGCCATTTTAATCA	179
17	ZMM1700	CATTAACACCATTACGCAAACA	TTTGGCAAAACTAGCAATGAA	258
18	ID0175	CAATTTTGATTTCTTTATCTATTTTCG	TCGAGTGCCCGAATTTTAAG	271
19	ZMM1353	GCCAAAACAAAGGATTCAAGA	TGAGCTTTGTGTGACCATGA	169
20	ID0145	ACCCTCCCTCCATGAATTTT	CCTCCATCTCATCTCATCCC	196
21	ZMM4803	TGCATGAGCTAAGGGAAAGG	TGGTGGCAATTTGCAAGTAA	268
22	ZMM6141	AAAAAGCAAAATCCATAATTTGA	TTGCCCCCTCAACTATTTG	167
23	ZMM3013	TGCCAGTTGGCATATACCATTA	GAGCCGGTCTGAAATTTATCC	216
24	ZMM2818	CGTGTGCCCAATATTTGAGTT	TCAACCTCCTCCCTACACAA	279
25	ZMM3223	CGATGGTTATTAAATTAAGTATTCGG	GACATTTGAAGCAAAGTGTATCG	279
26	ZMM1691	CTTGACCTGGAGTGTACGGC	GGATCAAACAGACACGAGCA	220
27	ZMM1851	TGACTCTTTCGATTTGGGCT	CGAAAAATACGGGCGTTACT	280

Source: Wei et al. [20], bp = base pairs.

4.2.3.2 Genotypic Data Analysis

The fragment sizes were determined using the ABI 3730 automatic sequencer. Data were analysed using the software GeneMarker V 2.2.0 to determine peak detection threshold levels that ranged from the minimum intensity of 500 and max intensity of 30,000. The 27 primers were used to detect the band sizes based on the peak detection thresholds, which were then scored using 1 to denote presence and 0 for absence. Genetic parameters, such as major allele frequency (M.A.F.), observed heterozygosity (Ho), expected heterozygosity (He), and the polymorphic information content (PIC) were calculated using Power Marker v3.2. Cluster analysis was carried out using a neighbor-joining (NJ) algorithm using the unweighted pair group method (UWPGM) in R software version 4.0 [25].

The population structure of the 100 sesame accessions was investigated using the Bayesian clustering method in STRUCTURE version 2.3.4 [27]. The length of the burn-in period and Markov Chain Monte Carlo (MCMC) were set at 20,000 iterations [28]. To obtain an accurate estimation of the number of populations, ten runs were performed for each K-value (assumed number of subpopulations), ranging from 1 to 10. Further, Delta K values were calculated, and the appropriate K value was estimated by implementing the method using CLUMPK [28]. The principal coordinate analysis was also used to deduce the genotypes' genetic structure using Darwin version 6.

4.3. Results

4.3.1. Genetic Variation and Mean Performance of Sesame Accessions

Combined ANOVA revealed significant ($p \leq 0.05$) entry x environment interaction for plant height, internode length, number of primary branches, number of secondary branches, distance from the base of the lowest branch to 1st capsule, and grain yield per hectare (see Section 3.3.1). Entries showed significant ($p \leq 0.05$) differences for days-to-50% flowering, days-to-75% maturity, plant height, internode length, number of secondary branches, number of seeds per capsule, distance from the base of the lowest branch to 1st capsule, and grain yield per hectare.

Based on grain yield performance, the top 10 best performing and the five bottom performing accessions are summarized in Table 4.2. The mean grain yield across locations was 0.48 ton ha^{-1}, and the mean thousand-seed weight was 2.9 g. The highest grain yield was recorded for entries such as:

Hirhir Kebabo Hairless-9 (1.01 ton ha^{-1}), Setit-3 (0.84 ton ha^{-1}), Orofalc ACC-2 (0.80 ton ha^{-1}), Hirhir Humera Sel-6 (0.78 ton ha^{-1}), ABX= 2-01-2 (0.74 ton ha^{-1}), and Setit-1 (0.73 ton ha^{-1}). These genotypes expressed high oil yields of 0.40, 0.40, 0.40, 0.39, 0.36, and 0.39 ton ha^{-1} than other test genotypes. The accessions Bawnji Fiyel Kolet, NN0056, Hirhir Humera Sel-8, NN-0068-1, and ACC-NS- 010 had the highest oil content of 55.6, 55.2, 54.7, 54.6, and 54.10% than other genotypes, respectively. The five bottom performing accessions in terms of grain yield were NN- 0183-3 (0.17 ton ha^{-1}), NN-0020 (0.24 ton ha^{-1}), NN-0108-2 (0.26 ton ha^{-1}), NN00136-1 (0.26 ton ha^{-1}), and NN-0143 (0.28 ton ha^{-1}) with low oil yield of 0.07, 0.12, 0.04, 0.13, and 0.15 ton ha^{-1}, in that order. These accessions yielded below-average grain and oil yields.

Table 4.2. Mean values for agronomic traits of 100 sesame genotypes of Ethiopia showing the top 10 and bottom 5 ranked entries based on grain yield (ton ha^{-1}) across two sites.

		Traits													
No	Entry name or designation	DF	DM	PH	INL	NPB	NSB	NCPP	NSPC	SHB	DFLBC	TSW	SYH	OYH	OC
	Top 10 entries														
1	Hirhir Kebabo Hairless-9	41	90	123.9	9.8	4	1	41	58	18.1	42.8	3.0	1.01	0.40	50.9
2	Setit-3	43	91	131.6	10.0	3	2	52	63	18.0	26.5	3.1	0.84	0.40	49.1
3	Orofalc ACC-2	42	89	103.9	8.3	3	2	36	56	13.8	22.4	3.1	0.80	0.40	52.5
4	Hirhir Humera Sel-6	43	92	114.8	9.7	4	4	48	61	27.2	38.1	2.9	0.78	0.39	53.9
5	ABX=2-01-2	44	95	133.2	8.7	4	3	40	65	18.9	40.9	2.7	0.74	0.36	48.9
6	Setit-1	40	88	125.6	7.9	4	2	39	56	21.9	38.9	3.3	0.73	0.39	53.8
7	ACC 205-180	45	97	126.9	10.5	3	2	40	55	32.5	42.0	3.1	0.72	0.38	53.1
8	ACC 203-616	45	94	106.8	8.5	3	1	37	54	25.5	39.0	2.6	0.69	0.35	51.7
9	NN-0029(2)	40	88	118.5	9.5	4	1	44	65	28.2	41.9	3.4	0.68	0.36	50.7
10	GA-002(3)	42	90	119	10.3	4	2	49	63	27.7	34.8	2.8	0.67	0.33	49.3
	Bottom 5 entries														
1	NN-0183-3	43	92	117.1	8.1	3	2	34	48	20.8	42.6	2.3	0.17	0.07	45.8
2	NN-0020	43	98	125.8	8.2	4	2	34	62	29.0	44.4	2.6	0.24	0.12	49.3
3	NN-0108-2	39	90	135.2	10.2	4	0	32	47	20.6	47.2	2.9	0.26	0.04	47.3
4	NN00136-1	43	97	99.6	10.2	3	2	42	44	26.1	47.4	3.0	0.26	0.13	47.5
5	NN-0143	42	94	132.2	10.3	4	3	30	55	27.2	39.7	3.2	0.28	0.15	48.3
	Mean	43	92	119.6	9.2	3.5	2	41	51	24.4	39.8	2.9	0.48	0.24	49.7
	CV (%)	5.03	3.61	13.30	13.52	19.21	38.58	29.42	20.92	29.74	20.44	78.06	35.26	90.13	NA
	R^2 (%)	72.87	70.33	68.59	86.27	85.16	82.97	77.76	70.65	77.22	85.95	60.98	82.15	66.42	NA
	LSD ($p \leq 0.05$)	3.03	4.65	21.78	1.73	0.96	0.88	16.74	14.67	10.16	11.33	3.23	0.24	0.32	NA

Note: Coefficient of variation (CV), Coefficient of determination (R^2), Not available (NA), Days-to-50% flowering (DF), Days-to-75% maturity (DM), Plant height (PH) (cm), Inter node length (INL) (cm), Number of primary branches per plant (NPB), Number of secondary branches per plant (NSB), Number of capsules per plant (NCPP), Number of seeds per capsule (NSPC), Stem height to 1st branch (SHB) (cm), Distance from lowest branch to 1st capsule (DFLBC)(cm), Thousand-seed weight (TSW) (g/1000 seed), Grain yield per

hectare (GYH) (ton ha^{-1}), Oil yield per hectare (OYH) (ton ha^{-1}), Oil content (OC) (%), Means in a column followed by the same letter are not significantly different at the 5% probability level of Tukey's Honestly Significant Difference.

4.3.2. Correlations of Yield and Yield Components

Phenotypic correlation coefficients for the studied traits are presented in Table 4.3. Grain yield was significantly and positively correlated with oil yield ($r = 0.99$; $p < 0.01$). Significant and positive correlations were also observed between grain yield and internode length ($r = 0.35$; $p < 0.01$), number of secondary branches ($r = 0.21$; $p < 0.01$), number of capsules per plant ($r = 0.18$; $p < 0.01$), number of seeds per capsule ($r = 0.17$; $p < 0.01$), stem height from base to 1st branch ($r = 0.16$; $p < 0.01$), and thousand-seed weight ($r = 0.23$; $p < 0.01$), whereas, significant and negative correlations were recorded between grain yield and distance from lowest branch to 1st capsule ($r = -0.29$; $p < 0.01$).

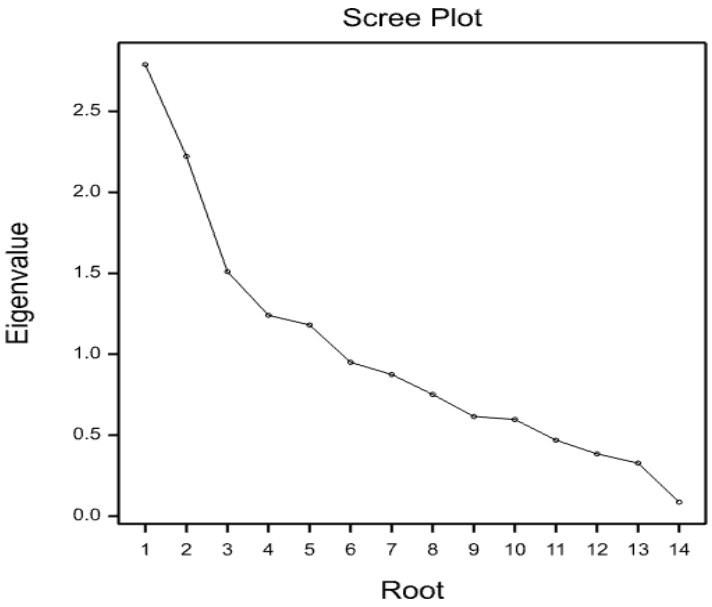
4.3.3. Principal Component Analysis

Principal component analysis (PCA) was computed to show each trait's contribution to the overall observed variation. A scree plot was generated to visualize the number of principal components. Overall, four principal components were identified with >1 Eigen values of which principal components 1 (PC1) and PC2 explained the highest proportion to the total variance (Figure 4.1). Principal component one (PC1) explained 19.9% to the total variation with OYH and GYH contributing the largest variation to PC1. Principal component two (PC2) accounted for 15.9% of the total variation, and DM, DF, DFLBC, and NPB were the most influential traits.

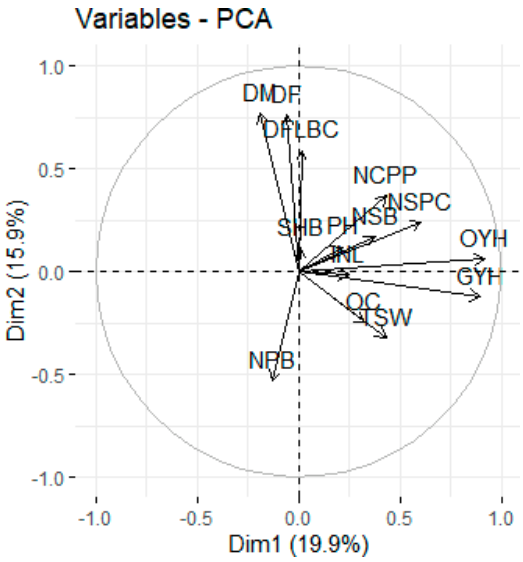
Table 4.3. Phenotypic correlations coefficients for assessed agro-morphological traits, oil content, and oil yield of 100 sesamecollections evaluated across two locations in Ethiopia.

Traits	DM	PH	INL	NPB	NSB	NCPP	NSPC	SHB	DFLBC	TSW	OC	OYH	SYH
DF	0.31**	0.05ns	0.14**	0.09ns	0.09ns	0.16**	-0.21**	0.05ns	-0.13**	-0.02ns	-0.11*	0.08ns	0.09ns
DM	1.00	0.07ns	0.08ns	-0.20ns	0.07ns	-0.10*	-0.12*	0.09ns	0.00ns	-0.15**	-0.06ns	-0.10*	-0.09ns
PH		1.00	0.09ns	0.06ns	0.12*	0.07ns	0.05ns	0.01ns	0.07ns	0.10*	-0.00ns	0.05ns	0.05ns
INL			1.00	-0.17**	0.21**	0.08ns	-0.08ns	0.38**	-0.45**	0.00ns	0.03ns	0.35**	0.35**
NPB				1.00	0.06ns	0.30**	0.08ns	-0.14**	0.25**	0.20**	0.05ns	-0.05ns	-0.06ns
NSB					1.00	-0.05ns	0.04ns	0.34**	-0.166**	-0.122*	0.051ns	0.214**	0.207**
NCPP						1.00	0.119*	-0.00ns	-0.01ns	0.16**	-0.01ns	0.17**	0.18**
NSPC							1.00	-0.07ns	0.21**	0.04ns	-0.08ns	0.15**	0.17**
SHB								1.00	-0.26**	-0.13**	0.02ns	0.16**	0.16**
DFLBC									1.00	-0.01ns	-0.02ns	-0.29**	-0.29**
TSW										1.00	0.05ns	0.23**	0.23**
OC											1.00	0.16**	0.06ns
OYH												1.00	0.99**

Note: * and ** denote significant difference at the 5% and 1% levels of probability, respectively; Non-significant (NS), Days-to-50% flowering (DF), Days-to-75% maturity (DM), Plant height (PH) (cm), Inter node length (INL) (cm), Number of primary branches per plant (NPB), Number of secondary branches per plant (NSB), Number of capsules per plant (NCPP), Number of seeds per capsule (NSPC), Stem height to 1st branch (SHB) (cm), Distance from lowest branch to 1st capsule (DFLBC)(cm), Thousand-seed weight (TSW) (g/1000 seed), Oil content (OC) (%), Oil yield per hectare (OYH) (ton ha^{-1}), Grain yield per hectare (GYH) (ton ha^{-1}).



(a)



(b)

Figure 4.1. Principal component analysis based on agro-morphological traits, oil content and oil yield of 100 sesame germplasm collections. Note: Figure (**a**) is a scree plot indicating eigenvalues and percentage variation explained by principal components; while Figure (**b**) is a biplot that indicatessesame traits projection on the first two principal components. Days-to-50% flowering (DF), Days-to-75% maturity (DM), Plant height (PH) (cm), Inter node length (INL) (cm), Number of primary branches per plant (NPB), Number of secondary branches per plant (NSB), Number of capsules perplant (NCPP), Number of seeds per capsule (NSPC), Stem height to 1st branch (SHB) (cm), Distancefrom lowest branch to 1st capsule (DFLBC)(cm), Thousand-seed weight (TSW) (g/1000 seed), Oil content (OC) (%), Oil yield per hectare (OYH) (ton ha^{-1}), Grain yield per hectare (GYH) (ton ha^{-1}), Dim1 = 19.9%, and Dim2 = 15.9%.

4.3.4. Genetic Polymorphism of the SSR Markers

The summary statistics describing the SSR markers are presented in Table 4.4. The major alleles frequency per locus ranged from 0.52 to 0.96, with a mean of 0.78 alleles per locus. The observed heterozygosity varied from 0.08 to 0.96, with a mean of 0.43. The unbiased expected heterozygosity (gene diversity) of the markers ranged from 0.08 to 0.5, with a mean of 0.30. The PIC values ranged from 0.07 (for markers ID0041, ID0175, and ZMM2818) to 0.37 (ZMM3261 and ZMM1189) with a grand mean value of 0.25.

Table 4.4. Genetic parameters estimated for 100 sesame genotypes using 27 SSR markers.

Locus	Genetic parameter			
	MAF	He	Ho	PIC
ID0046	0.72	0.40	0.56	0.32
ZMM1043	0.75	0.38	0.51	0.31
ZMM3261	0.59	0.48	0.82	0.37
ID0041	0.96	0.08	0.08	0.07
ZMM5015	0.79	0.34	0.43	0.28
ZMM4664	0.60	0.48	0.80	0.36
ZMM1809	0.86	0.24	0.28	0.21
ZMM2321	0.90	0.18	0.20	0.16
ZMM5358	0.62	0.47	0.77	0.36
ID0068	0.86	0.25	0.29	0.22
ZMM3312	0.56	0.49	0.89	0.37
ZMM1033	0.76	0.37	0.49	0.30
ZMM1189	0.52	0.50	0.96	0.37
ZMM2202	0.89	0.20	0.22	0.18
ZMM1637	0.68	0.44	0.65	0.34
ZMM4645	0.81	0.31	0.39	0.26
ZMM1700	0.95	0.10	0.10	0.09
ID0175	0.96	0.08	0.08	0.07
ZMM1353	0.94	0.12	0.13	0.11
ID0145	0.77	0.36	0.47	0.29
ZMM4803	0.95	0.10	0.11	0.10
ZMM6141	0.75	0.38	0.51	0.31
ZMM3013	0.69	0.43	0.63	0.34
ZMM2818	0.96	0.08	0.08	0.07
ZMM3223	0.82	0.30	0.36	0.25
ZMM1691	0.73	0.39	0.54	0.32
ZMM1851	0.90	0.18	0.20	0.16
Mean	**0.78**	**0.30**	**0.43**	**0.25**

MAF = Major allele frequency, He = Unbiased expected heterozygosity (gene diversity), Ho = Observed heterozygosity, PIC = Polymorphic information content.

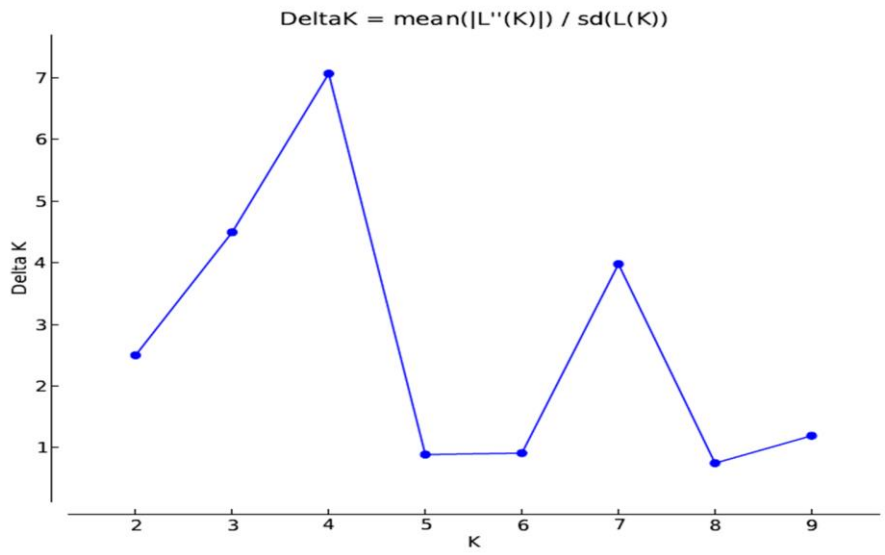
4.3.5. Population Structure Analysis

Structure analysis revealed four populations amongst the 100 sesame entries (Figure 4.2b, Table 4.5). Sixty-three genotypes were allocated to the four populations, whereas 37 genotypes were admixtures with no specific membership (Table 4.5). Population I consisted of 24 genotypes collected from the following regions: Amhara (17 collections), Tigray (3), Afar (3), and Oromia (1). Population II had 13 genotypes initially collected from the Amhara region (8), Afar (4), and Tigray (1). Population III comprised nine genotypes sourced from the Amhara (5 accessions) and Tigray (4) regions. Population IV consisted of 17 genotypes sourced from Tigray (10 accessions), Amhara (6), and Afar (1) regions.

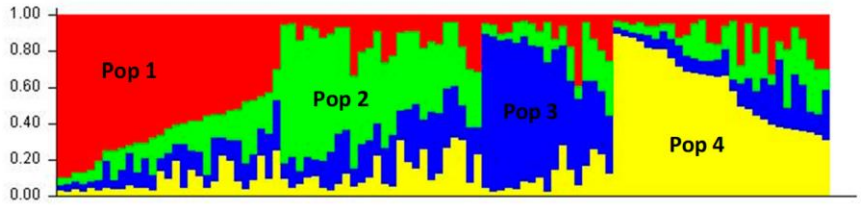
Table 4.5. Genetic clusters and their member entries, the proportion of the membership, mean expected heterozygosity, and fixation index based on structure analysis of 63 sesame entries with 27 SSR markers.

Population	Entries	Membership %	Expected heterozygosity	Mean fixation index
I	ACC NS-031E63, Hirhir Nigara 1st Sel-2, Hirhir Kebabo Hairless Sel-6, NN-0183-3, GXT=85(28-2), ABXT-85-Sel-2-1, ABXC-50402, NN-0129-2, ACC-203-020, Gonjam Azene (Aleka), NN-0026, Tejareb Kokit Sel-3, NN0027, NN0009, NN-0088-2 Bawnji Sel-2, G-02, Endelemi kirem sel-2, NN0016-1, NN0038-1, NN-0052, NN0071, NN0064-1	24	0.15	0.39
II	Hirhir Baeker-Sel-3, NN0025, BCS-0041, Orofalc ACC-2, ABX=2-01-2, Bering Bawany, ACC-203-612, NN-0143, NN-0146, NN-0044-2, NN-0018-2, NN-0029-1, NN0056	13	0.22	0.23
III	Hirhir kebabo hairless sel-2, Hirhir kebabo hairless-9, ACC-200-064-1, HIRHIR NIGARA 1ST SEL-1, Bawnji Fiyel Kolet, NN0104, Bawnji Maksegnt, Bawnji Flwha Sel-2, Hirhir Filwha Large Seeded	9	0.29	0.02
IV	Hirhir Humera Sel-6, Humera-1, Setit-1, Setit-3, Hirhir Sel-2, Hirhir Kebabo Hairless-Sel-7, Hirhir Humera Sel-8, NN0036-1, Hirhir Kebabo Early Sel-1, Hirhir Adgeshu Sel -8, Morgo-Sel-P=13, NN-0020 ACC-203-610, NN0001-2, NN01-13, Gojam Azene(Yohans Sel-1), NN0031	17	0.20	0.29

(a)



(b)



(c)

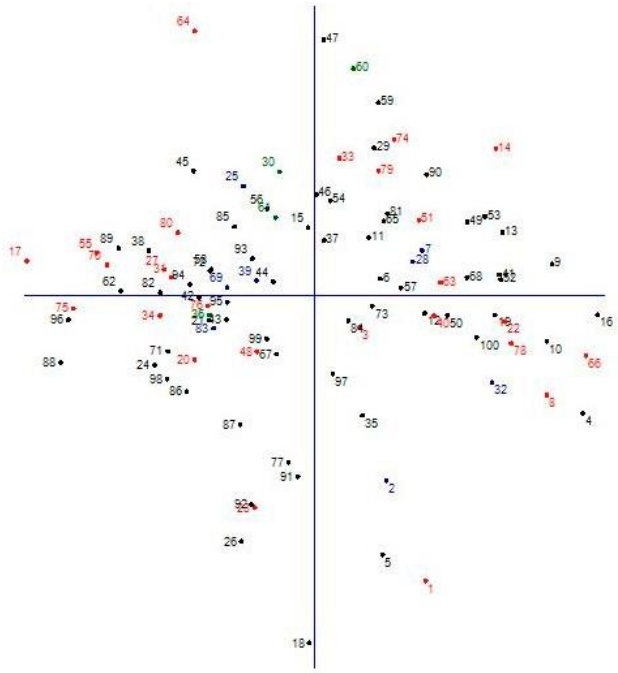


Figure 4.2. Subpopulation inference among100 sesame entries based on 27 SSR markers: (**a**) Delta K estimation based on the Evanno procedure, (**b**) Sub-populations for the best delta K value of four. Pop 1, 2, 3, and 4 denote Populations 1, 2, 3, and 4, respectively, and (**c**) principal coordinate clustering of genotypes.

Entries allocated in population I had good branching ability (NN-0052, NN-0029-1, and GXT = 85(28-2)), many seeds per capsule (Gojam Azene (Aleka), and ABXT-85-Sel-2-1), and significant oil yield (NN-0026), and seed oil content (NN0064-1 and NN0071).

Population II genotypes were early maturing with tall plants. Some population II accessions had a significant number of seeds per capsule (NN0025 and ABX = 2-01-2), and grain yield (Orofalc ACC-2, and ABX = 2-01-2). In addition, population II comprises accessions such as NN0056, Hirhir Baeker-Sel-3, and Orofalc ACC-2 with good oil content. Genotypes allocated in population III were early maturing with taller plants. These accessions were outstanding in thousand-seed weight (e.g., Hirhir Filwha Large Seeded), grain yield (Hirhir Kebabo Hairless-9), oil yield (Hirhir Kebabo Hairless-9), and seed oil content (Hirhir Kebabo Hairless-9).

Genotypes allocated in population IV were also early maturing with taller plants. Some genotypes within this group also had remarkable seeds per capsule (Setit-3 and NN-0020), better thousand-seed weight (3.4 g), higher seed and oil yields (0.84 and 0.40 ton ha−1), and oil content (54.7%). To develop new breeding populations possessing desirable economic traits new crosses could be developed between the selected parents. Hence genotypes Orofalc ACC-2 (from population II), Hirhir Filwha Large Seeded (population III), and Setit-3, Hirhir Humera Sel-6 (population IV) are ideal candidates with comple- mentary traits for production and further breeding. However, the principal coordinate analysis assigned the 100 genotypes into admixture groups with an inconclusive structure (Figure 4.2c).

4.3.6. Cluster Analysis of 100 Sesame Genotypes

Cluster analysis involving 100 sesame genotypes resolved two clusters, and each cluster was further partitioned into two sub-clusters (Figure 4.3). Cluster I consisted of 49 accessions and one improved variety sourced from the following regions: Amhara (37 accessions), Tigray (5 accessions and one improved variety), Afar (6 accessions), and Oromia (1 accession). Cluster II contained 50 diverse genotypes, of which 28 accessions were from Amhara, while 13 accessions, one landrace and 3 improved varieties from Tigray, 2 accessions (from Afar), 2 accessions (Oromia), and 1 accession (Gambela). Likewise, as observed from the population structure analysis, all the two main clusters and their respective sub-branches had genotypes with the potential for optimum grain yield and high oil content.

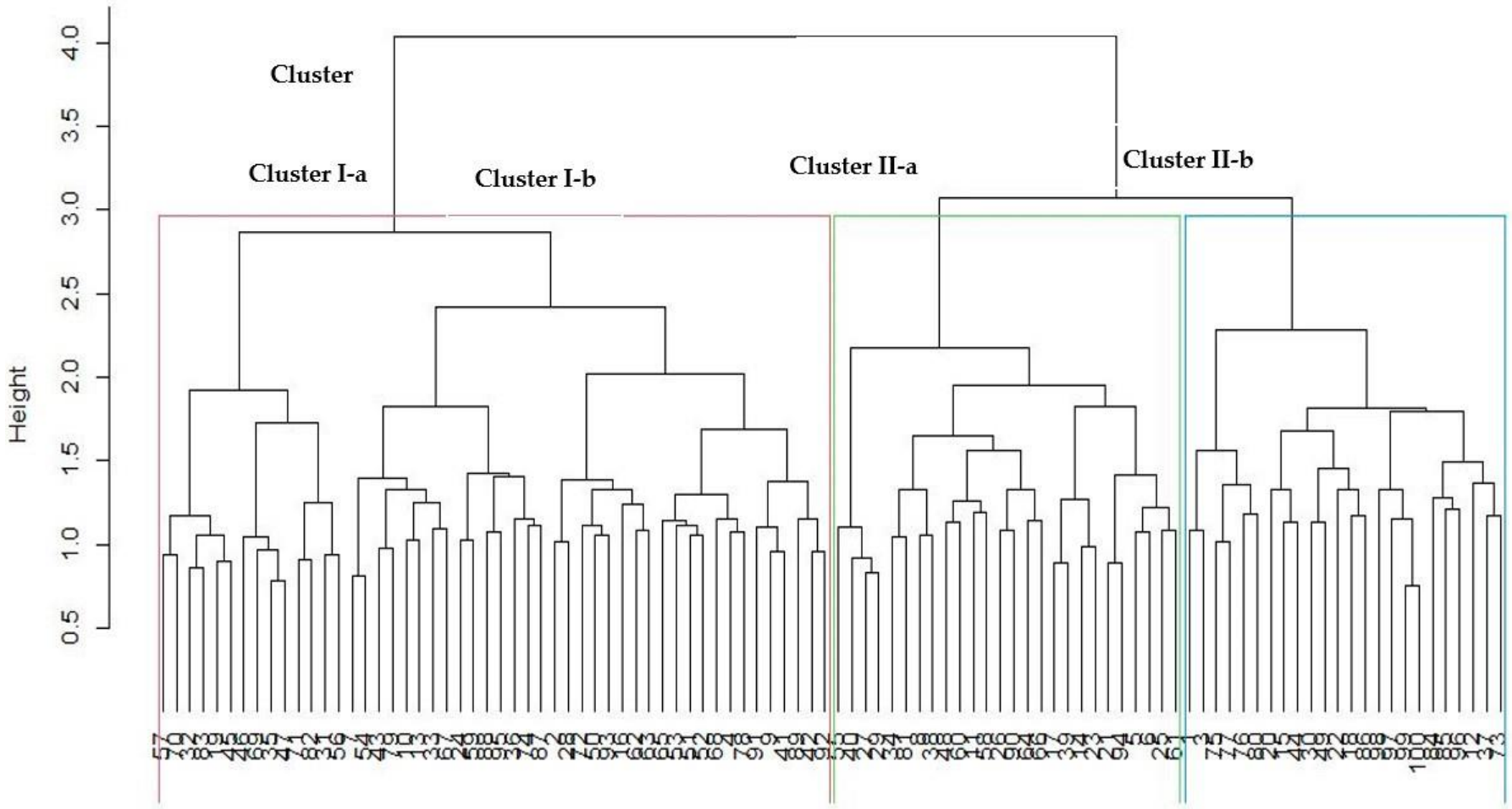


Figure 4.3. Dendrogram based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA) showing the genetic relationship among 100 sesame entries using 27 SSR markers. Note: see codes of entries in Table 3.1, Section 3.2.2.

4.4. Discussion

4.4.1. Genotypic Variation and Mean Performance for Seed and Oil Yields, and Yield-Component Traits

Assessment of genetic diversity among crop genetic resources is essential to identify candidate accessions possessing desirable traits, including yield and quality attributes. The current study evaluated the genetic variation present among 100 accessions of sesame through rigorous field phenotyping and polymorphic SSR markers as a preliminary step to select genetically complementary parental accessions for breeding.

The test genotypes showed significant ($p \leq 0.05$) variation for grain yield and yield components (Table 4.2, Section 3.3.1). This suggests that the germplasm pool contains vital phenotypic traits for sesame improvement through hybridization and selections. The test genotypes were sourced from five historically sesame-growing regions in Ethiopia. Given the long agricultural history and sesame production of the collection areas, it is expected that the test genotypes have adapted and evolved under local conditions through natural selection. This caused genetic differentiation of the studied sesame accessions for grain and oil yields and important yield-contributing agronomic traits. For example, the present study identified and selected sesame genotypes such as Hirhir Kebabo Hairless-9 and Setit-3 with high grain yields of >0.8 tons ha^{-1} and higher oil yields of 0.40 ton ha^{-1}. The selected genotypes, which are locally referred to as Humera types, are known for their unique quality associated with product aroma and taste [29]. The selected genotypes expressed higher grain yield which is above the mean yield of 0.68 tons ha^{-1} currently recorded in Ethiopia using traditional varieties.

4.4.2. Traits Associations

Sesame seed and oil yields are low in Ethiopia due to a lack of high-yielding varieties. These results in low financial returns for producers and processors across the sesame value chains. To improve selection response and genetic gains for economic traits, selection of highly heritable yield-contributing traits associated with seed and oil yields may be targeted in sesame improvement programs. The strong and positive correlation between seed and oil yield among the studied sesame genotypes implied both traits could be improved simultaneously in the present population. Weak correlations observed between grain yield with yield-related traits, including internode length, number of secondary branches, number of capsules per plant, stem height from base to first branch, and thousand seed weight would provide a low selection response for grain yield.

Similarly, oil yield exhibited low correlations with internode length, the number of secondary branches per plant, and thousand-seed weight implying reduced selection response for grain yield via these traits. Oil content showed poor associations with agro-morphological traits hindering direct selection. Despite the low and poor associations between seed and oil yields and oil content with yield-related agronomic traits, the present study revealed wide phenotypic variation among the studied sesame populations for several traits. These are valuable traits for future sesame phenotypic analysis, selection, and improvement in Ethiopia. Moreover, the assessed germplasm was diverse for seed and oil yields and oil content. This aided identification and selection of sesame genotypes such as Hirhir Kebabo Hairless-9, Setit-3, Orofalc, Hirhir Humera Sel-6, and Setit-1 with high seed and oil yields as useful germplasm to design and develop improved cultivars. Furthermore, sesame genotypes with relatively higher oil content, including Hirhir Humera Sel-6, Setit-1, ACC 205–180, and Orofalc ACC-2 are suitable candidates for developing new breeding populations with higher oil yield and content.

The traits accounting for the significant variation observed in the first two PCs will be important for selection. Nevertheless, 53.4% of the total variation was not explained by the PCA, probably due to the limited number of test locations used in the study. Baraki et al. [30] identified three PCs, which explained 88.49% of the total variation in 13 sesame genotypes in Ethiopia for eight agronomic traits. They reported that grain yield and oil content were the largest contributors to the explained variation in PC1. However, the number of genotypes used in the present study was much larger and ideal for genetic variability and cluster analyses. Hence, there is a need to assess the test accessions across multiple test environments and using effective molecular markers to complement the phenotypic data.

4.4.3. Genetic Diversity and Population Structure of Sesame Germplasm Based on SSR Markers

SSR markers are amongst the useful genomic resources to complement phenotypic data for effective selection. The present study recorded a mean major alleles frequency per locus of 0.78 among the sesame population (Table 4.4), which was much higher than values of 0.41 and 0.17 reported by [21,31] using 23 and 21 SSR among 129 Korean and 25 Ghanaian sesame genotypes, respectively. Variation in alleles frequency is attributable to genotypic differences and the number of SSR markers used in the genetic analysis [32–34]. In general, markers having the largest number of alleles had the lowest MAF, unlike those with the lowest number of alleles. The mean observed heterozygosity of 0.43 reported in the present study is lower than the value of 0.56 reported by [31] when assessing 25 sesame genotypes using 21 SSR markers. This study's observed heterozygosity was higher than values

of 0.23, 0.01, and 0.12 reported by [19,21,22] when assessing 50, 129, and 36 sesame genotypes using 10, 23, and 10 SSR markers, respectively. The mean expected heterozygosity (He = 0.30) recorded in the present study (Table 4.4) was lower than values of 0.72 and 0.34 reported by [21,22] when evaluating 129 and 36 sesame accessions using 23 and 10 SSR markers, respectively. The higher heterozygosity recorded in the present study suggested that the Ethiopian sesame populations have a high genetic variation for effective selection.

Existence genetic diversity was confirmed by population structure analysis, which revealed four distinct populations comprising genotypes collected from different regions in Ethiopia. Most released entries (Humera-1, Setit-1, and Setit-3) were grouped in subpopulation 4. Wei et al. [20] and Asekova et al. [21] reported two and three distinct populations from 94 and 129 sesame accessions sampled from Chinese and Korean collections using 44 and 23 SSR markers. The higher gene fixation index of 0.39 in population I comprising genotypes collected from Amhara, Tigray, Afar, and Oromia regions suggest higher genetic differentiation attributable to high gene flow among these regions. Conversely, the low gene fixation index observed in population III, which comprises accessions sourced from the Amhara and Tigray regions, indicated low differentiation. This may be due to gene flow through germplasm exchange between sources of collections. The exchange of planting material regardless of geographical distances might be attributed to a low degree of differentiation in sesame populations observed in the current study.

Cluster analysis identified two major clusters and four sub-clusters, revealing genetic diversity among the assessed sesame entries (Figure 4.3). Asekova et al. [21] grouped 129 sesame genotypes into two clusters using 23 SSR markers. In the present study, the genotypes' clustering patterns did not correspond to the predefined population structure based on the collection regions. This may be because genotypes gathered from different regions belong to the same gene pool or may have similar ancestral relationships [35]. Conversely, William et al. [36] reported that genetic dissimilarity among test genotypes could arise due to the diverse ancestral origin, high gene flow caused by cross-pollination and possible gene or chromosomal mutation. In this study, some sesame genotypes collected from different regions were grouped into the same clusters, such as Hirhir Kebabo Hairless Sel-6 (Tigray) and Gojam Azene (Yohans Sel-1) (Amhara), and ACC-NS-007(2) (Oromia) and GA-002(3) (Gambela) which were found in cluster I and II, respectively. In agreement with the current study, Zhang et al. [37] reported that geographical separation did not affect genetic distance among 24 sesame genotypes. Ganesamurthy et al. [38] reported that geographical separation does not affect the genetic differentiation of germplasm. Therefore, a key indicator of genetic diversity is not

necessarily the geographical origin of germplasm collections. The exchange of genetic materials among farmers and traders in the regions might contributed to high gene flow and lack of genetic differentiation. Barnaud et al. [39], suggested that farmers' selections and management practices affect genetic diversity patterns.

To develop new breeding populations possessing desirable agronomic traits, especially high grain and oil yields, crosses could be made between distantly related and complementary genotypes selected from different clusters. For instance, for improved grain and oil yields, entries such as Setit-3, Orofalc ACC-2, Hirhir Humera Sel-6, ACC-NS-007(2), Hirhir Kebabo Hairless-9, and ACC 205-180 were selected. These genotypes are localized in sub-cluster II-a and sub-cluster II-b, with excellent grain and oil yields.

4.5. Conclusions

The current study determined the extent of genetic diversity among 100 sesame germplasm collections from Ethiopia using phenotypic traits and simple sequence repeat (SSR) markers to select distinct and complementary parents for breeding. The test genotypes exhibited significant phenotypic variation for key agronomic traits including grain yield, oil content, and oil yield, which were underpinned by their genetic diversity. The sesame genotypes were differentiated into four major populations based on the model-based population structure analysis. The moderate heterozygosity and fixation index among the genotypes suggests that the genotypes have distinct grouping patterns desirable for breeding. Based on wide genetic divergence, the following genotypes were selected for use in future sesame breeding programs: Hirhir Humera Sel-6, Setit-3, Hirhir Kebabo Hairless Sel-4, Hirhir Nigara 1st Sel-1, Humera-1, Orofalc ACC-2, and Hirhir Kebabo Early Sel-1 (selected from subgroup II-a), and Hirhir kebabo hairless-9, NN-0029(2), NN0068-2, Hirhir Filwha Large Seeded, and Bawnji Fiyel Kolet, (from subgroup II-b). Progeny development and field evaluation by combining ability analysis are recommended among the selected parents to establish heterotic groups for sesame pre-breeding.

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Chapter 5. Genetic diversity and population structure of sesame (*Sesamum indicum* L.) genotypes for seed oil and fatty acid compositions

Abstract

Knowledge of the genetic profiles of diverse germplasm collections of sesame using seed oil, fatty acid contents, and molecular markers is a prerequisite to develop market-preferred cultivars with quantity and quality oil. The objective of this study was to determine the genetic diversity and relationships among Ethiopia's sesame germplasm collections using seed oil content and fatty acid compositions and diagnostic simple sequence repeat (SSR) markers to select genetically complementary and promising parental lines for breeding. The contents of the seed oil and fatty acids of 100 lines grown under field conditions were determined using the near-infrared reflectance spectrometry. Twenty-seven polymorphic SSR markers were used to assess the genetic profile of the test lines and complement the seed oil and fatty acid data. The SSR markers revealed that the mean gene diversity and polymorphic information content were 0.30 and 0.25, respectively. Population structure analysis identified four major heterotic groups. Based on higher oil content and desirable fatty acid compositions and SSR markers the following superior and complementary lines such as: Hirhir Kebabo Hairless Sel-6 (from sub-cluster I-b), Hirhir Humera Sel-8 and NN0058-2 (sub-cluster II-a) and Bawnji Fiyel Kolet (sub-cluster II-b) are identified for sesame breeding programs or production globally.

Keywords: Fatty acids, genetic diversity; SSR markers; seed oil content, *Sesamum indicum*

5.1. Introduction

Sesame (*Sesamum indicum* L.; 2n= 26), belonging to the family Pedaliaceae is one of the oldest and highly valuable oilseed crops globally (Ashri, 2010). Its domestication dates back some 5,500 years ago in the Harappa Valley of India, mainly as an oilseed crop (Bedigian and Harlan, 1986). Sesame is regarded as the queen of oilseed crops due to its high quantity and quality oil and commercial value (Dossa et al. 2018). The seed oil content of sesame varies from 40.80 to 60.30%, with a mean of 53.00%, the highest value compared with other oilseed crops (Dossa et al. 2018). Sesame oil has about 85% unsaturated fatty acids (e.g., oleic acid, linoleic acid and linolenic acid), which are beneficial to human health. Consumption of sesame oil is believed to be minimising the risks of cardiovascular diseases, cancer, brain and liver damages (Yen et al. 1990; Yol et al. 2015). The major fatty acids present in the sesame oil include oleic acid (35.90-47.00%), linoleic acid (35.60-47.60%), palmitic acid (8.70-13.80%), and stearic acid (2.10-6.40%) (Weiss 1983; Uzun et al. 2002; Elleuch et al. 2007). Also, trace amounts of linolenic acid (1.38–2.19%) and arachidic acid (0.10-0.70%) are present in the seed oil.

Sesame is an economically important crop widely traded in local, regional and international markets (Myint et al. 2020). Global sesame consumption is steadily increasing due to high demands related to its unique nutritional values such as higher contents of vitamins (e.g., A and E), minerals, fibre, healthy fatty acids [e.g., oleic acid, linoleic acid], carbohydrate (~13.5%), and protein (~24%)] (Myint et al. 2020). Furthermore, population pressure, urbanisation and the changing lifestyle have increased the global demand for sesame products (Myint et al. 2020).

About 70% of the world's sesame seed is processed to produce food oil, while the seedcake is used to prepare livestock meals (Myint et al. 2020). The total annual human consumption of sesame is about 65% and 35% in the form of food oil and grain (e.g., as a garnish, snack, and flavouring agent in some foods), respectively (Morris 2020). In 2019 the total world sesame oil production was 1,286,741.00 tons. China with a total annual production of 563,637.00 tons, Myanmar (154,600.00 tons), India (96,800.00 tons), Japan (53,257.00 tons), Nigeria (45,690.00 tons) and Turkey (38,300.00 tons) are the major global sesame oil producers in 2019 (FAOSTAT 2019). Globally, a total of 76,140.00 tons of sesame oil was traded with a monetary value of 331.2 billion USD in 2019 (FAOSTAT 2019). The top sesame oil-exporting countries were China (with 16,829.00 tons), Japan (9,244.00 tons), India (8,593.00 tons), Lebanon (6,214.00 tons), and Mexico (5,997.00 tons) (FAOSTAT 2019).

In sub-Sharann Africa, Sudan, the United Republic of Tanzania, Nigeria, Burkina Faso, Ethiopia, and Mozambique are sesame grain's major producers and exporters (FAOSTAT 2019). In Ethiopia, sesame is the leading oil crop that occupies some 45.7% of the total production area, followed by niger seed (*Guizotia abyssinica* [L.f.] Cass.) and groundnut (*Arachis hypogea* L.) (CSA 2019). Sesame is Ethiopia's second major export cash crop that contributes significantly to gross domestic product and foreign currency earnings (Gebremedhn et al. 2019). Globally, Ethiopia is the 8th largest sesame producer with a total annual grain production of 262,654 tons after Sudan (1,210,000 tons), China (936,104 tons), Myanmar (744,498 tons), India (689,310 tons), Tanzania (680,000 tons), and Nigeria (510,000 tons), Burkina Faso (374,703 tons) (FAOSTAT, 2019).

The average grain yield of sesame in Ethiopia is 0.6 ton ha^{-1}, which is far below the attainable yield of the crop, up to 4.00 t/ha. The mean yield of sesame is variable across the major producing countries such as in Lebanon (3.52 tons ha^{-1}), Saudi Arabia (2.53 tons ha^{-1}), Afghanistan (2.16 tons ha^{-1}), Tajikistan (2.12 tons ha^{-1}), Israel (2.05 tons ha^{-1}), Uzbekistan (1.77 tons ha^{-1}) and China (1.62 tons ha^{-1}) (FAOSTAT 2019). Sesame production and productivity in East Africa, including Ethiopia have stagnated because of lack of high-yielding and well-adapted varieties (Were et al. 2006). In the region, sesame production relies on unimproved landrace varieties selected by farmers. These varieties have intrinsic oil quality characteristics, such as unique aroma and taste by consumers. Hence, there is a need to breed lines with high oil quantity and quality for local and export markets, and for diverse production environments.

Ethiopia is the centre of origin and diversity for the cultivated sesame (Mehra 1967; Bedigian 1981; Seegeler 1983; Mahajan 2007). More than 5000 genetically diverse sesame accessions are maintained by the Ethiopian Biodiversity Institute (EBI) (Woldesenbet et al. 2015). The accessions, including landraces and introductions, are ideal sources of genetic variation to initiate a competitive breeding program focused on new variety design and deployment based on seed oil quantity and quality. These genetic resources need to be systematically evaluated for seed oil, fatty acid contents, and genetic composition to develop improved cultivars that meet the demands of sesame clients and value chains. The product profile of the new sesame varieties should include high oil content and well balanced fatty acid compositions such as palmitic, stearic, oleic, linoleic, and linoleic acids. The local sesame germplasm provides the genetic foundation for breeding programs to select agronomically suitable and locally well-adapted varieties with a suite of nutritional values for human health. There are limited studies that reported on oil quantity and quality of sesame using Ethiopia's sesame germplasm. Wang et al. (2012) used high-performance liquid chromatography (HPLC) to determine the sesamin and

sesamolin contents of 215 sesame lines from the Chinese core collection. The authors found a broad genetic variation for sesamin (0.05-11.05 mg/g) and sesamolin (0-10 mg/g) contents. Dossa et al. (2018) reported the presence of great variation for seed oil content, oleic acid content, linolenic acid content and protein content amongst 139 African and Asian sesame germplasm collections using the near-infrared reflectance spectrophotometry (NIRS) scanning. Further, Biglar et al. (2012) analysed the seed oil content and fatty acid profiles of 5 Iranian sesame cultivars using a gas chromatography and reported a considerable variation. Broad variation in oleic acid, linoleic acid, linolenic acids, palmitic acid, myristic acid, capric acid, and lauric acids were reported by Wacal et al. (2019) using a total fat determination unit in sesame collections of Japan.

Molecular markers are highly reliable genetic tools that can complement phenotypic characterization for breeding (Jones et al. 2009). The following markers are widely used in genetic diversity analysis of crop species: amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR), simple sequence repeat (SSR), and single nucleotide polymorphisms (SNPs). These marker systems have provided useful and complementary data revealing genetic diversity, relationships and population structure of sesame germplasm collections (Laurentin et al. 2006; Abdellatef et al. 2008; Gebremichael et al. 2011; Wei et al. 2016; Dossa et al. 2016; Asekova et al. 2018; Araújo et al. 2019, Basak et al. 2019; Teklu et al. 2021). The SSR markers have been widely used in analysing the sesame genetic diversity, population structure and heterotic groups. The markers are highly preferred for detecting higher degrees of polymorphism, reproducibility, allelic variation, and their abundance in the genomes (Wei et al. 2016; Dossa et al. 2016; Asekova et al. 2018; Araújo et al. 2019). Wei et al. (2016), Dossa et al. (2016), Asekova et al. (2018) and Araújo et al. (2019) reported considerable genetic diversities of sesame populations using the SSRs.

Nutrient profiles, including oil content and fatty acid compositions, show wide variability in sesame germplasm collections, providing opportunities for breeding for enhanced oil quantity and quality (Dossa et al. 2018). Previous studies have reported the presence of considerable variation for seed oil content in sesame genetic resources from Ethiopia (Gidey et al. 2012; Teklu et al. 2014; Hika et al. 2014, 2015). Limited studies assessed the genetic diversity of sesame accessions in Ethiopia using SSRs (Gebremichael et al. 2011) and inter simple sequence repeat (ISSR) (Woldesenbet et al. 2015) markers. However, the previous reports did not fully represent Ethiopia's sesame collection, and the markers used were relatively few. Therefore, there is a need for a comprehensive assessment of the genetic diversity of oil content and fatty acid compositions present in the Ethiopian sesame germplasm pool

using a greater number of accessions representing the diverse growing areas through oil content and fatty acid compositions and polymorphic SSR markers. Hence, the objective of this study was to determine the genetic diversity and relationships among Ethiopia's sesame germplasm collections using seed oil content and fatty acid compositions and diagnostic simple sequence repeat markers to select genetically complementary and promising parental lines for breeding.

5.2. Materials and methods

5.2.1. Plant materials

This study evaluated 100 genetically diverse sesame genotypes, including 95 accessions, one landrace, and four improved varieties. The details of the germplasm collections used in the study are presented in Table 3.1, Section 3.2.2.

5.2.2. Study site and experimental design

The 100 sesame lines were evaluated in northwestern Ethiopia at the Humera site (14°150 N, 36°370 E) to sample seeds for seed oil quality and quantity analysis as described in Sections 3.2.1 and 3.2.3.

5.2.3. Determination of oil content and fatty acid profiles

Oil content and fatty acid composition were determined at Wuhan city in China using the Near-Infrared Spectroscopy (NIR) (FOSS, model DS2500, Hillerod, Denmark). All mature and well-rounded seeds were separated, and true-to-type seeds were used for accurate analysis. For each genotype 3 g of seed was sampled and labelled to determine the contents of oil and the profiles of palmitic acid (16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3), all values expressed in percentage. The NIR analyzer was programmed with different calibration models for dark (black, brown and black) and light (white, light white, brown, and light brown) seeds (Dossa et al. 2018). All the sample seeds were irradiated with near-infrared monochromatic light, and the reflectance spectrum (log10 1/R) was recorded from 1100 to 2500 nm at a wavelength interval of 2 nm, according to Dossa et al. (2018). During the NIR analysis, the room temperature was maintained at 25 °C. Each sample was run in duplicate analyses, and if the difference between the two values was >2, the analysis was repeated to increase the accuracy of the analysis (Dossa et al. 2018).

5.2.4. Genotyping using SSR markers

The seeds harvested from the Humera site were prepared for genotypic analysis at the Oil Crops Research Institute (OCRI)-the Chinese Academy of Agricultural Sciences (OCRI-CAAS), China.

5.2.4.1. DNA extraction, primer selection, polymerase chain reaction, and electrophoresis analysis

The details of DNA extraction, primer selection, polymerase chain reaction, and electrophoresis analysis are described in Section 4.2.3.1.

5.2.5. Data analysis

5.2.5.1. Oil content and fatty acid compositions

Data collected between the two analyses did not show significant differences in oil content and the major fatty acid profiles. Hence, the two data sets were pooled to conduct a one-sample t-test analysis to discern the significant difference ($P < 0.05$). The t-test analysis was conducted using the Statistical Package for the Social Sciences (SPSS) software version 20 (SPSS 2020).

The seed oil content and fatty acid profiles data were subjected to correlation analysis to determine the magnitude of associations using R software version 4.0 (R Core Team 2000). The oil content and fatty acid compositions were subjected to principal component analysis to determine the magnitude of variation attributable to the various components and identify the most discriminative parameter for selection. Principal component analysis was done using R software version 4.0 (R Core Team 2000). The test genotypes were subjected to cluster analysis for oil content, and fatty acid contents based on Euclidean distances using SAS procedure CLUSTER (SAS Institute, 2018). This allowed the determination of the genetic relatedness and classification of the assessed genotypes into respective genetic groups. Bi-plot analysis was computed to infer genotype association regarding the seed oil content and fatty acid profiles using R software version 4.0 (R Core Team 2000).

5.2.5.2. SSR markers data analysis

Genetic parameters, such as major allele frequency (MAF), observed heterozygosity (Ho), expected heterozygosity (He), and the polymorphic information content (PIC) and other parameters were estimated using appropriate software as described in Section 4.2.3.2.

5.3. Results

5.3.1. Seed oil content and fatty acid compositions of sesame genotypes

The one-sample t-test analysis revealed significant ($P \leq 0.05$) differences among the test genotypes for seed oil content and all the assessed fatty acid profiles (Table 5.1). This suggests differential responses of the assessed sesame genetic resources for production or selection programs with desirable oil and fatty acid compositions.

The mean oil content and fatty acid compositions of sesame genotypes are summarized in Table 5.1. The grand mean of the oil content across the studied genotypes was 49.84%, varying from 44.30 to 55.60%. The mean values for palmitic and stearic acid contents were 9.10% and 5.01%, respectively. The highest oil content of 55.6% was recorded for entry Bawnji Fiyel Kolet at, followed by NN0056 (55.2%), Hirhir Humera Sel-8 (54.7%), NN-0068-1 (54.6%), ACC-NS-010 (54.1%), and NN0015 (54.0%). Gojam Azene at 44.30% exhibited the lowest seed oil content recorded in the study. The oleic acid content of the assessed genotypes ranged from 36.8 to 48.8%, with a mean of 42.9%, while the linoleic acid content varied from 36.6 to 47.1%, with a mean of 41.7%. Genotypes that recorded the highest oleic acid content were NN0058-2 (48.80%), Tejareb Kokit Sel-3 (48.2%), Setit-2 (47.5%), ACC-203-020 (47.3%), Hirhir Humera and NN-0088-2 (46.7%). Hirhir Humera Sel-8 recorded the lowest oleic acid content of 36.76%. Some of the assessed genotypes had a linoleic acid content of > 45%, including Hirhir Humera Sel-8 (47.14%), Hirhir Humera Sel-6 (46.00%), ACC-205-299 (45.71%), ACC-NO-041 (45.67%), and Hirhir Kebabo Hairless Sel-4 (45.56%).

Table 5.1. Mean values of the assessed sesame genotypes for the contents of seed oil and fatty acids

Entry number	Genotype name or designation	Fatty acid compositions (%)†					
		OC (%)	C16:0	C18:0	C18:1	C18:2	C18:3
1	Hirhir Kebabo Hairless Sel-2	50.5	8.76	4.95	46.64	38.21	0.29
2	GXT=85(28-2)	51.00	9.46	5.05	40.26	43.95	0.28
3	Hirhir Kebabo Hairless-9	50.9	8.90	4.92	45.35	39.53	0.28
4	NN-0068-1	**54.6**	9.08	**4.84**	41.5	43.26	0.24
5	NN-0108-2	47.3	9.06	5.14	41.38	42.35	0.35
6	NN-034	47.7	9.21	5.12	40.91	44.04	0.30
7	BCS-0041	46.9	9.21	5.07	43.82	41.02	0.34
8	ACC-031-5-14	47.90	8.85	5.03	44.79	40.27	0.31
9	NN-0129-2	44.60	9.82	5.12	39.72	44.63	0.36
10	ACC-203-020	46.80	8.66	5.11	**47.34**	37.93	0.36
11	NN-0038-2	50.80	9.39	4.97	43.34	41.15	0.28
12	Bawnji Fiyel Kolet	**55.60**	9.27	**4.81**	40.04	44.19	0.28
13	Gojam Azene (Aleka)	**44.30**	9.05	**5.36**	41.77	40.99	**0.39**
14	Hirhir Humera Sel-6	53.90	9.64	4.86	37.79	**46.00**	**0.23**
15	Bawnji Gobate	48.90	8.88	5.04	45.86	38.89	0.30
16	Shwarobit (83)	50.60	9.00	5.02	40.9	43.27	0.33
17	Humera-1	52.50	9.34	4.91	40.82	43.6	0.28
18	ACC-202-950	48.20	9.01	5.01	40.68	43.32	0.35
19	NN-0026	49.10	9.04	5.07	41.52	43.5	0.28
20	ACC-NO-041	51.60	9.00	**4.73**	38.74	**45.67**	0.33
21	ACC-203-612	48.40	8.78	5.04	46.44	38.55	0.34
22	ACC-200-064-1	48.20	8.75	5.07	43.95	41.29	0.34
23	Setit-1	53.80	9.60	4.88	38.51	45.35	0.25
24	Tejareb Kokit Sel-3	49.90	8.98	5.01	**48.21**	**36.56**	0.31
25	Orofalc ACC-2	52.50	9.14	4.90	45.29	38.73	0.26
26	NN-0022	46.8	9.01	5.06	42.89	41.93	0.32
27	Setit-3	49.10	**10.23**	5.06	38.85	44.98	0.33
28	ABX=2-01-2	48.90	8.86	5.10	42.42	42.41	0.30
29	NN-0020	49.30	8.89	5.03	43.40	41.69	0.30
30	ACC-NS-010	**54.10**	**8.51**	4.91	40.11	44.97	0.31
31	Hirhir Sel-2	51.80	9.38	4.97	39.89	44.54	0.27
32	Abxt-85-Sel-2-1	45.40	8.74	5.18	45.6	39.81	0.35
33	Setit-2	46.10	8.84	5.12	**47.54**	37.49	**0.39**
34	Hirhir Kebabo Hairless-Sel-7	53.00	9.42	4.90	44.52	39.88	0.24
35	NN-0143	48.30	9.03	5.08	45.13	39.72	0.28
36	ACC NS-031	44.90	9.04	5.17	40.67	42.53	**0.38**
37	NN-0029(2)	50.70	8.89	4.92	45.95	38.92	0.28
38	NN-0054	48.80	9.16	5.12	44.58	40.08	0.26
39	Morgo-Sel-P=13	53.20	9.21	4.87	42.45	41.88	0.27
40	Hirhir Humera Sel-8	**54.70**	9.85	**4.83**	**36.76**	**47.14**	0.28
41	Tejareb Girar	53.80	9.19	4.94	40.64	43.75	0.27
42	NN0027	47.90	8.67	5.06	44.85	40.47	0.32
43	NN0009	46.50	9.54	5.13	44.46	39.98	0.37
44	ACC-203-610	51.80	8.86	4.94	44.37	40.4	0.30
45	NN-0146	47.40	**8.63**	5.00	45.61	39.53	0.34
46	NN-0044-2	50.60	9.17	4.99	45.41	39.36	0.29
47	NN-0018-2	49.90	9.15	5.04	41.48	42.81	0.33
48	Hirhir Nigara 1st Sel-1	50.40	8.88	5.00	44.3	40.76	0.30
49	NN00136-1	47.50	9.01	5.19	41.49	41.95	0.35
50	NN-0088-2	47.30	**8.62**	5.08	**46.71**	38.39	0.35
51	Hirhir Baeker-Sel-3	53.40	**8.63**	4.92	40.49	44.66	0.33
52	NN0068-3	51.20	8.98	4.96	43.33	41.72	0.26
53	NN0074-3	47.40	8.91	5.16	41.76	43.39	0.31

Table 5.1. (Continued).

Entry number	Genotype name or designation	Fatty acid compositions (%)†					
		OC (%)	C16:0	C18:0	C18:1	C18:2	C18:3
54	NN0036-1	49.40	8.71	5.02	42.26	42.61	0.32
55	Hirhir Kebabo Hairless Sel-4	53.50	9.20	4.92	38.9	**45.56**	0.24
56	NN0001-2	50.90	9.18	4.94	43.35	41.31	0.29
57	Bawnji Sel-2	46.50	9.18	4.94	43.35	41.31	0.29
58	NN0058-2	48.20	8.71	5.07	**48.80**	36.77	**0.39**
59	G-02	48.70	9.47	4.84	43.99	40.68	0.34
60	ACC-NS-007(2)	52.40	9.81	5.05	42.71	41.14	0.32
61	GA-002(3)	49.30	8.94	4.92	44.54	39.99	0.28
62	Endelemi Kirem Sel-2	52.50	9.37	5.11	43.73	40.35	0.31
63	ACC-205-299	46.30	8.76	5.01	39.27	**45.71**	0.29
64	Hirhir Kebabo Early Sel-1	53.70	9.03	5.17	40.17	43.37	0.36
65	NN0016-1	49.20	9.26	4.85	40.24	44.17	0.28
66	Hirhir Adgeshu Sel -8	49.20	9.79	4.95	43.93	40.31	0.34
67	NN0015	**54.00**	9.32	5.04	42.88	41.48	0.30
68	NN01-13	50.60	9.06	4.91	43.01	41.39	0.29
69	Bering Bawany	48.80	9.48	5.03	43.38	40.70	0.29
70	Hirhir Nigara 1st Sel-2	47.90	9.13	5.05	42.89	41.86	0.32
71	Gojam Azene (Yohans Sel-1)	53.10	8.94	5.11	42.50	42.43	0.33
72	NN0038-1	48.60	9.45	4.91	41.02	43.18	0.29
73	NN0104	51.80	9.28	5.13	44.85	39.73	0.33
74	Hirhir Kebabo Hairless Sel-6	50.30	**8.31**	4.92	41.27	43.91	0.29
75	ACC 205-180	53.10	8.87	4.99	44.04	41.1	0.29
76	Hirhir	45.80	9.34	4.93	41.44	42.58	0.25
77	ACC 203-616	51.70	9.03	5.00	40.32	43.39	**0.38**
78	NN0025	46.60	8.73	4.91	45.93	38.73	0.28
79	NN-0183-3	45.80	9.22	5.08	45.35	39.53	0.36
80	Hirhir Humera	49.50	8.86	5.13	**46.71**	38.50	**0.38**
81	NN0031	50.00	8.91	5.08	44.20	40.82	0.29
82	NN0061	50.10	8.83	4.99	44.97	39.9	0.33
83	ABXC-50402	48.70	9.33	4.94	43.12	41.44	0.32
84	NN0021	51.50	9.18	5.06	44.36	40.15	0.32
85	NN0079-1	52.40	9.05	5.02	42.76	42.12	0.30
86	ACC 202-333	40.00	9.04	5.03	40.30	39.02	0.33
87	NN-0052	46.80	9.02	5.20	40.70	42.98	0.37
88	NN-0029-1	47.40	9.02	5.09	40.50	43.52	0.35
89	Teiahir Sanja Sel-6	50.90	9.19	5.02	44.35	40.27	0.29
90	ACC-202-358	48.50	9.23	5.00	44.5	40.28	0.33
91	NN0032	52.60	8.93	4.96	45.3	39.42	0.3
92	NN0071	51.20	9.15	4.96	41.97	42.29	0.31
93	NN0064-1	51.10	9.11	5.03	41.36	43.57	0.29
94	NN0056	**55.20**	9.08	**4.82**	40.76	44.06	0.27
95	NN-01-03	50.00	9.43	5.03	43.67	40.69	0.31
96	NN0032-2	49.50	9.32	5.03	42.96	41.51	0.31
97	Bawnji Maksegnt	48.30	9.71	5.09	45.67	37.82	0.32
98	Bawnji Flwha Sel-2	47.60	9.03	4.98	39.23	44.32	0.36
99	NN0068-2	49.80	9.02	4.93	39.6	44.47	0.35
100	Hirhir Filwha Large Seeded	48.10	8.90	5.11	44.13	40.8	0.34
	t-statistics, (df=98)	189.55	284.50	486.76	172.53	184.37	83.67
	Standard deviation (SD)	2.59	0.32	0.10	2.45	2.23	0.04
	Signficant value (p=0.05)	**	**	**	**	**	**
	Mean	49.84	9.10	5.01	42.85	41.69	0.31
	Minimum	44.30	8.31	4.73	36.76	36.56	0.23
	Maximum	55.60	10.23	5.36	48.80	47.14	0.39

** denote significance at the 1% probability level

† OC, Oil content; C16:0, Palmitic acid; C18:0, Stearic acid; C18:1, Oleic acid; C18:2, Linoleic acid; and C18:3, Linolenic acid;

Bold-face values denote selected genotypes with the highest values, while entries highlighted in red expressed

the lowest values.

5.3.2. Correlations of oil content and fatty compositions

Phenotypic correlation coefficients for the seed oil content and fatty acid profiles of the 100 sesame genotypes are presented in Table 5.2. Highly significant negative correlations were observed for the contents of oil and stearic acid ($r = -0.83$; $p < 0.01$) and linolenic acid ($r = -0.81$; $p < 0.01$), whereas relatively higher and positive correlations were recorded between stearic and linolenic acid contents ($r = 0.61$; $p < 0.01$). Significant positive correlations were recorded between seed oil content and linoleic acid content ($r = 0.39$; $p < 0.01$).

Table 5.2. Phenotypic correlations coefficients for the assessed seed oil content and fatty acid compositions (%) of 100 sesame genotypes.

Traits†	OC	C16:0	C18:0	C18:1	C18:2	C18:3
OC	1.00	0.14ns	-0.83**	-0.35**	0.39**	-0.81**
C16:0		1.00	-0.12ns	-0.37**	0.28**	-0.19*
C18:0			1.00	0.26**	-0.31**	0.61**
C18:1				1.00	-0.98**	0.19*
C18:2					1.00	-0.24*
C18:3						1.00

†OC, Oil content; C16:0, Palmitic acid; C18:0, Stearic acid; C18:1, Oleic acid; C18:2, Linoleic acid; and C18:3, Linolenic acid; * and **, denote significant at the 5% and 1% probability levels, respectively; ns, non-significant.

5.3.3. Principal component analysis

Principal component analysis (PCA) was performed to ascertain each trait's contribution to the overall observed variation in seed oil and fatty acid compositions. Overall, three principal components were identified with Eigenvalues ≥1 explaining 81.00 % of the total variation for the assessed traits (Table 5.3 and Figure 5.1). The first two principal components (PC1) and PC2 explained the highest proportion to the total variance. PC1 explained 44.00% of the total variation, with oil content and stearic acid contributing the most significant variation to PC1. While PC2 accounted for 22.00% of the total variation and oleic and linoleic acids were the most influential traits. Figure 5.1 is a bi-plot showing the sesame genotypes and traits projection based on the first two principal components. The two principal components or dimensions accounted 59.20% (Dm1= 36.6% and Dm2= 22.6%) to the total variation. The bi-plot delineated the test genotypes and traits across the four quadrants. For instance, entries 97 and 62 recorded the highest oleic acid and linoleic acid contents, respectively and situated in quadrant II.

Table 5.3. Eigenvalues, explained variation, cumulative variation (%) and principal components (PCs) for oil content and fatty acid compositions amongst 100 sesame germplasm collections.

Traits†	PC1	PC2	PC3
OC	**-0.49**	-0.34	0.08
C16:0	-0.22	0.30	0.08
C18:0	**0.44**	0.35	-0.09
C18:1	0.41	**-0.53**	0.03
C18:2	-0.42	**0.48**	-0.04
C18:3	0.42	0.39	0.11
Eigenvalue	3.11	1.56	1.02
Explained variation (%)	44.00	22.00	15.00
Cumulative explained variation (%)	44.00	66.00	81.00

†OC, Oil content; C16:0, Palmitic acid; C18:0, Stearic acid; C18:1, Oleic acid; C18:2, Linoleic acid; and C18:3, Linolenic acid.

Bold-faced values in a column denote the most influential traits corresponding to the principal component.

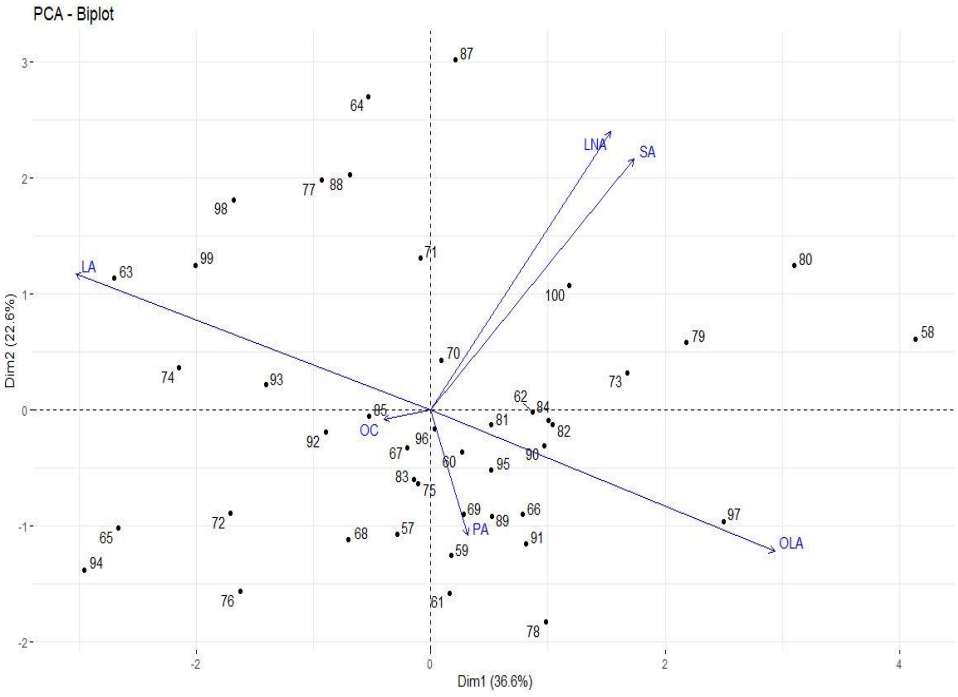

Figure 5.1. A bi-plot depicting the distribution of 100 sesame genotypes using seed oil and fatty acid contents based on two principal components or dimensions (Dm1= 36.6% and Dm2= 22.6%). Note OC, Oil content, PA, Palmitic acid, SA, Stearic acid, OLA, Oleic acid, LA, Linoleic acid, and LNA, Linolenic acid. See codes of the test genotypes in Table 3.1, Section 3.2.2.

5.3.4. Cluster analysis of sesame genotypes based on oil content and fatty compositions

Based on the contents of oil and fatty acids, cluster analysis resolved the 100 sesame genotypes into two main clusters (Table 5.4). Each cluster was further partitioned into two sub-clusters revealing genotype differentiation for selection. Cluster I comprised 61 genotypes and one landrace (Hirhir). Amongst the accessions, 45 were originally collected from Amhara, 12 from Tigray, 3 from Afar, and one each from Oromia and Gambela administrative regions in Ethiopia. Cluster II contained 34 genotypes of which 19 were sourced from Amhara region, 8 from Tigray, 5 from Afar and 2 from Oromia regions. Furthermore, Cluster II comprises four improved varieties (Humera-1, Setit-1, Setit-2, and Setit-3) sourced from the Tigray region.

Most of the genotypes allocated in Cluster I had intermediate to high oil content (50.90 to 55.20%) but low to intermediate oleic (36.76 to 44.64%), linoleic (36.77 to 40.80%), and linolenic acids (0.24 to 0.29%). Cluster I genotypes Hirhir Humera Sel-8, NN0015, and NN0056 had relatively higher oil content (≥54%). Most accessions in this cluster recorded oleic acid of <45%, except NN-0088-2 and Hirhir Humera, which had >46%. Furthermore, Cluster I comprised accessions such as Hirhir Humera Sel-8, Hirhir Kebabo Hairless Sel-4 and ACC-205-299, which had higher linoleic contents of > 45%. Genotypes allocated in Cluster II had low to high oil content (44.30 to 55.60%) but relatively higher oleic acid (39.89 to 48.21%), linoleic acid (38.55 to 46.00%), and linolenic acid (0.29 to 0.39%). Most test genotypes in Cluster II recorded oil contents of <50%, except Bawnji Fiyel Kolet, NN-0068-1, Setit-1, Hirhir Kebabo Hairess-Sel-7, Hirhir Humera Sel-6, and ACC-NS-010, which had ≥53%. Genotypes Hirhir Kebabo Hairless Sel-2, ACC-203-020, ACC-203-612, Tejareb Kokit Sel-3, and Setit-2 expressed >46% of oleic acid contents. Genotypes Hirhir Humera Sel-6, ACC-No-041, and Setit-1 were grouped in Cluster II and expressed linoleic compositions of higher than 45%.

Table 5.4. Distribution of the 100 genotypes collections based on oil content and fatty acid compositions.

Germplasm	Source (regions or research centre in Ethiopia)	Cluster and collection name			
		I		II	
		I-a	I-b	II-a	II-b
Accessions	Amhara Region	NN0016-1, NN0015, NN01-13, Gojam Azene(Yohans Sel-1), NN0038-1, NN0104, ACC 203-616, NN0025, NN-0183-3, NN0031, NN0061, NN0021, NN0079-1, NN-0052, NN-0029-1, Teiahir Sanja Sel-6, ACC-202-358, NN0032, NN0071, NN0064-1, NN0056, NN-01-03, NN0032-2, Bawnji Maksegnt, Bawnji Flwha Sel-2, NN0068-2, Hirhir Filwha Large Seeded, ACC 202-333	Tejareb Girar, NN0027, NN0009, ACC-203-610, NN-0146, NN-0044-2, NN-0018-2, NN00136-1, NN-0088-2, NN0068-3, NN0074-3, NN0036-1, NN0001-2, Bawnji Sel-2, NN0058-2, G-02, Endelemi Kirem Sel-2,	ACC-203-612, Tejareb Kokit Sel-3, NN-0022, NN-0020, NN-0143, NN-0029(2), NN-0054	NN-0068-1, NN-0108-2, NN-034, NN-0129-2, ACC-203-020, NN-0038-2, Bawnji Fiyel Kolet, Gonjam Azen(Aleka), Bawnji Gobate, Shwarobit(83, ACC-202-950, NN-0026
	Tigray Region	Hirhir Adgeshu Sel -8, Hirhir Nigara 1st Sel-2, Hirhir Kebabo Hairless Sel-6, ACC 205-180, Hirhirh Humera	Hirhir Humera Sel-8, Hirhir Nigara 1st Sel-1, Hirhir Baeker-Sel-3, Hirhir Kebabo Hairless Sel-4, ACC-205-299, Hirhir Kebabo Early Sel-1	ACC-200-064-1, Hirhir Sel-2, Hirhir Kebabo Hairless-Sel-7	Hirhir kebabo Hairless sel-2, Hirhir kebabo Hairless-9, ACC 031-5-14, Hirhir Humera Sel-6, ACC-NO-041,
	Afar Region	Bering Bawany, ABXC-50402	Morgo-Sel-P=13	Orofalc ACC-2, ABX=2-01-2, ABXT-85-SEL-2-1	GXT=85(28-2), BCS-0041
	Oromia Region	-	ACC-NS-007(2)	ACC-NS-010, ACC NS-031	
	Gambela Region		GA-002(3)	-	-
Landrace	Tigray Region	Hirhir	-		
Improved varieties	Humera Agricultural Research Center	-	-	Setit-2, Setit-3	Humera-1, Setit-1
	Sub-total	36	26	17	21
	Total		100		

5.3.5. SSR markers characterization

The summary of genetic parameters based on the tested SSR markers are presented in Table 4.4, Section 4.3.4. The major alleles frequency per locus ranged from 0.52 (for markers ZMM1189) to 0.96 (ZMM2818), with a mean of 0.78 alleles per locus. The observed heterozygosity had a mean value of 0.43, varying from 0.08 to 0.96. The average expected heterozygosity value was 0.30 and ranged from 0.08 to 0.5. The polymorphic information content values of the markers varied from 0.07 (markers ID0041, ID0175, and ZMM2818) to 0.37 (ZMM3261, ZMM3312 and ZMM1189), with a mean of 0.25. The magnitude of the PIC values determines the informativeness of the markers for genetic diversity analysis.

5.3.6. Population structure analysis

The Bayesian-based analysis of population structure using SSR markers showed that the log-likelihood at K = 4 was optimal to group the subset of 100 sesame accessions into four genetically distinct subpopulations (Figure 4.2a, Section 4.3.5).

5.3.7. Cluster analysis of 100 Sesame accessions

In agreement with the seed oil and fatty acid compositions (Table 5.4), the cluster analysis based on SSR markers resolved two main clusters and two sub-clusters (Figure 4.3, Section 4.3.6). Cluster I consisted of 49 accessions and one improved variety sourced from the following regions: 37 accessions (from Amhara), 5 accessions and one improved variety (Tigray), 6 accessions (Afar), and one accession (Oromia). Cluster II was the most divergent, which comprised 50 genotypes sourced from Amhara (28 accessions), Tigray (13 accessions, one landrace, and three improved varieties), Afar (two accessions), and Oromia (two accessions), and Gambela (one accession). Similar to the population structure analysis, all the two main clusters and their respective sub-branches had genotypes with high oil and fatty acid contents.

Genotypes allocated in Cluster I had intermediate (50.00 to 53.8%) to high (54.00 to 54.6%) oil (e.g. NN-0068-1, NN0015, Gojam Azene [Yohans Sel-1], Tejareb Girar, and Hirhir Baeker-Sel-3), high oleic acid (>45%) (Tejareb Kokit Sel-3, Setit-2, and NN-0088-2), high linoleic acid (>44%) (ACC-205-299, Hirhir Baeker-Sel-3, Hirhir Sel-2, and NN0016-1) and high linolenic acid (>0.38) (Gojam Azene and ABX=2-01-2 contents.

Cluster II genotypes recorded higher oil, oleic and linoleic acid contents. Accessions Bawnji Fiyel Kolet, NN0056, Hirhir Humera Sel-8, and ACC-NS-010 had relatively higher oil content (>54%). The majority of genotypes in this cluster recorded oleic acid content of <45%, except NN0058-2, Hirhir Humera, Hirhir Kebabo Hairless Sel-2, and ACC-203-612, which had >46%. In addition, cluster II comprises genotypes such as Hirhir Humera Sel-8, Hirhir Humera Sel-6, ACC-NO-041, Hirhir Kebabo Hairless Sel-4, and Setit-1 with a relatively higher linoleic acid content (>44%) and higher linolenic acid content recorded on some accessions: NN0058-2 (0.39%), ACC 203-616 (0.38%), and Hirhir Humera (0.38%).

Crosses should be made between parental lines selected from different clusters with positive values to develop new breeding populations with desirable oil content and fatty acid compositions. Hence accessions Hirhir Kebabo Hairless Sel-6 (from sub-cluster I-b), Hirhir Humera Sel-8 and NN0058-2 (sub-cluster II-a) and Bawnji Fiyel Kolet (sub-cluster II-b), are ideal candidates with complementary fatty acid profiles for further breeding.

5.4. Discussion

5.4.1. Oil content and fatty acid compositions

The test genotypes showed significant ($p \leq 0.05$) variation for seed oil content and fatty acid profiles (Table 5.1). This suggests that the germplasm pool contains vital seed oil content and fatty acid profiles for sesame improvement through hybridization and selections. Oil content and fatty acid compositions of sesame genotypes for assessed traits are summarized in Table 5.1. The mean oil content across the studied genotypes was 49.84%, which was similar to 50.1% reported by Biglar et al. (2012). However, Dossa et al. (2018) reported a relatively higher mean oil content of 53.00%. In addition, Agidew et al. (2021) reported significantly higher oil content (53.2–58.2%) in the Ethiopian sesame germplasm. The mean palmitic and stearic acid content were 9.10 and 5.1% in that order (Table 5.1). In line with this, Park et al. (2015) reported a mean values of 9.9 and 5.8% for palmitic and stearic acid contents, respectively, in sesame accession from Korea. Contrary to the present study, Biglar et al. (2012) reported low palmitic acid (9.6%) and stearic acid (4.7%) contents in world collection of sesame. Agidew et al. (2021) reported higher palmitic and stearic acid contents of 10.2 and 6.15%, respectively, in the Ethiopian collections. The highest oil content was recorded for entries Bawnji Fiyel Kolet (55.6%), NN0056 (55.2%), Hirhir Humera Sel-8 (54.7%), NN-0068-1 (54.6%), ACC-NS-010 (54.1%) and NN0015 (54.0%). The oil content of the identified genotypes was higher than 31.00 to 48.00% of previously evaluated sesame germplasm in East Africa, including Kenya, Tanzania and Uganda (Were et al. 2006). Cultivation of these genotypes is vital for export market standards and to meet the quality attributes of

the confectionary industry globally. The oleic acid content ranged from 36.7 to 48.8 % (with a mean of 42.9%), which was higher than the mean oleic acid content of 38.10% and lower than the mean value of linoleic acid content of 41.70% in the present study reported by Dossa et al. (2018). Also, Biglar et al. (2012) reported a mean value of oleic acid content of 43.3% (range of 32.7 to 53.9%) in world sesame collection. Park et al. (2015) reported that oleic acid content ranged from42.0 to 43.0%, which is lower than the present study. Conversely, Agidew et al. (2021) reported relatively lower oleic acid (37.2 to 38.9%) and higher linoleic acid (42.5 to 44.3%) contents in the Ethiopian sesame collections. Genotypes Tejareb Kokit Sel-3 (48.2%), Setit-2 (47.5%), ACC-203-020 (47.3%), both Hirhir Humera and NN-0088-2 (46.7%) expressed the highest oleic acid content. Hirhir Humera Sel-6, ACC-NO-041, Setit-1, Hirhir Humera Sel-8, Hirhir Kebabo Hairless Sel-4 and Hirhir Kebabo Early Sel-1 with a linoleic acid content of > 45% were identified in the present study. The unsaturated fatty acids (C18:1 and C18:2), constitute the major fatty acids in sesame oil (84.0%) (Wei et al. 2015). Cultivating sesame genotypes with high oleic and linolic acid composition is beneficial to human health by minimising the risks of cardiovascular diseases, cancer, and brain and liver damage (Yen et al. 1990; Yol et al. 2015).

5.4.2. Traits associations

In the present study, oil content was negatively correlated with oleic acid content and corroborated with the findings of Dossa et al. (2018). This suggests that breeding sesame simultaneously for high oil and oleic acid contents would be difficult. A moderately high and positive correlation was recorded between stearic and linolenic acid contents, indicating both traits could be improved simultaneously among the studied sesame genotypes. Oleic acid content was negatively correlated with linoleic acid content (Table 5.2) and corroborated with the findings of Park et al. (2015). Similarly, Dossa et al. (2018) reported a negative correlation coefficient between sesame oleic and linoleic acid contents. Therefore, improvement of oil content among the studied sesame genotypes should be based on direct selection of high oil and fatty acid content genotypes and transgressive segregants in future improvement programs.

Identifying and selecting sesame genotypes with high oil content and fatty acid compositions is important in utilizing the germplasm in sesame breeding programs. Dossa et al. (2018) identified two PCs, which explained 79.43% of the total variation in 139 sesame genotypes in Africa and Asia for oil content, protein, and fatty acid compositions. They reported that linolenic acid and protein content were the largest contributors to the explained variation in PC1, whereas oil content and oleic acid content were in PC2. In the present study, the main contributors to the observed phenotypic variation were oil content, stearic acid, oleic and linolic acids with high loading coefficients in PC1 and PC2.

5.4.3. Cluster analysis of 100 sesame accessions

Cluster analysis identified two major clusters and four sub-clusters, revealing genetic variation among the assessed sesame genotypes (Table 5.4). Dossa et al. 2018 grouped 139 sesame genotypes into two clusters using oil, protein, and fatty acid compositions. In the present study, some sesame genotypes collected from different regions were grouped in the same cluster, such as Hirhir Humera Sel-8 (Tigray), Hirhir Filwha Large Seeded (Amhara), GA-002(3) (Gambela) and ABX=2-01-2 (Afar) and ACC NS-031 (Oromia). Therefore, the geographic origin of germplasm collections is not necessarily a key indicator of genetic diversity. The exchange of genetic materials among farmers and traders in the regions may contribute to genetic variation across different regions. For improved oil content and oleic, linoleic, and linolenic acids content the ideal genotypes for future crosses are situated in Clusters I and II, which possessed candidates with excellent oil content and oleic, linoleic, and linolenic acids composition.

5.4.4. Genetic diversity and population structure of sesame germplasm based on SSR markers

Simple sequence repeats markers are useful genomic resources to complement phenotypic data for effective selection. In the present study the major alleles frequency per locus recorded a mean value of 0.78 among the sesame genotypes (Table 4.4, Section 4.3.4), which was much higher than that reported by Asekova et al. (2018) and Adu-Gyamfi et al. (20190). The genotypic differences and the number of SSR markers used in the genetic analysis are attributable to variation in allele frequency (He et al. 2011; Baraket et al. 2011; Jifar et al. 2020). The average observed heterozygosity value of 0.43 reported in the present study is higher than the findings of Gebremichael et al. (2011); Asekova et al. (2018), and Araújo et al. (2019), who reported values of 0.23, 0.01, and 0.12 in sesame respectively. This study's observed heterozygosity was lower than the findings of He et al. (2011), who reported a value of 0.56 in sesame. The mean expected heterozygosity recorded in the present study (He = 0.30) (Table 4.4, Section 4.3.4), which was lower than the values of 0.72 and 0.34 reported by Asekova et al. (2018) and Araújo et al. (2019) using 23 and 10 SSR markers among 129 and 36 sesame accessions, respectively. The higher heterozygosity recorded in the present study suggested that the Ethiopian sesame populations have a high genetic variation for selection. In the present study, the higher heterozygosity recorded suggested that the Ethiopian sesame populations have a high genetic variation for selection.

The population structure revealed four populations (Figure 4.2b, Section 4.3.5). Using 44 and 23 SSR markers, Wei et al. (2016) and Asekova et al. (2018) found two populations among 94 and 129 sesame genotypes from China and Korea, respectively. Only 63 genotypes were structured into the four

populations. Nonetheless, the test accessions sourced from one region were distributed across the different populations. This indicates that geographical separation does not affect the genetic differentiation of sesame genotypes. Results showed that the four sub-populations comprised genotypes collected from different sources, although most of the released genotypes (Humera-1, setit-1 and 3) were grouped in subpopulation IV, except setit-2 was admixture.

The expected heterozygosity was 0.15, 0.22, 0.29 and 0.20 in subpopulation 1, 2, 3, and 4, respectively (Table 4.5, Section 4.3.4). The level of genetic differentiation among the subpopulations was measured by estimating the fixation index (FST). The results showed that subpopulation 1 with a higher FST of 0.39 was more differentiated than subpopulations 2, 3 and 4, with FST values of 0.23, 0.02, and 0.20. The greater gene fixation index of 0.39 in population I, which included accessions from the Amhara, Tigray, Afar, and Oromia regions, suggests that these regions have more genetic differentiation due to substantial gene flow. In contrast, the low gene fixation index found in population III, which includes accessions from the Amhara and Tigray regions, revealed a lack of differentiation. This could be attributed to gene flow from collection to collection via germplasm exchange.

The UPGMA cluster analysis identified two major clusters and four sub-clusters (Figure 4.3, Section 4.3.6). Using 23 SSR markers, Asekova et al. (20018) divided 129 sesame genotypes into two clusters. The genotype clustering patterns in this study did not match the intended population structure based on the collection regions. This could be because genotypes from similar places are from the same gene pool or have similar ancestral ties (Mulualem et al. 2018). On the other hand, William et al. (2019) suggested that genetic dissimilarity between test genotypes could be generated by a variety of ancestral origins, significant gene flow driven by cross-pollination, and probable gene or chromosomal mutation. Some sesame genotypes collected from different regions were grouped in the same cluster in the current study. This result corroborated the findings of Zhang et al. (2012a) among 24 sesame genotypes in China. This indicating that geographical separation does not affect the genetic differentiation of germplasm (Ganesamurthy et al. 2010). As a result, substantial gene flow and lack of genetic differentiation result from the exchange of genetic materials among farmers and traders in the regions. Farmers' selections and management practices affect genetic diversity patterns (Barnaud et al. 2008).

Parental lines with complementary traits selected from different clusters could be utilized to develop new breeding populations possessing desirable oil content and fatty acid compositions. Accordingly, Hirhir Kebabo Hairless Sel-6 (from sub-cluster I-b), Hirhir Humera Sel-8 and NN0058-2 (sub-cluster II-

a) and Bawnji Fiyel Kolet (sub-cluster II-b) are some of the accessions that might be considered desirable parents. These clusters comprised ideal candidates with high oil, oleic, linoleic, and linolenic contents for production and further breeding.

5.5. Conclusion

The current study determined the genetic diversity and relationships among Ethiopia's sesame germplasm collections using seed oil content and fatty acid compositions and diagnostic simple sequence repeat (SSR) markers to select genetically complementary and promising parental lines for breeding. The test genotypes showed wide variation for seed oil content and fatty acid compositions. The mean oil content of the assessed lines was 49.84% ranging from 44.30 to 55.60%. The oleic acid content ranged from 36.70 to 48.80%, with a mean of 42.90%, followed by linoleic acid (36.60 to 47.10%, mean 41.70%). The SSR markers revealed that the mean gene diversity and polymorphic information content were 0.30 and 0.25, respectively, indicating that the assessed sesame germplasms were diverse for selection. Population structure analysis identified four major heterotic groups useful for selection. Based on higher oil content and desirable fatty acid compositions and SSR markers the following superior and complementary lines were selected: Hirhir Kebabo Hairless Sel-6 (from sub-cluster I-b), Hirhir Humera Sel-8 and NN0058-2 (sub-cluster II-a) and Bawnji Fiyel Kolet (sub-cluster II-b). The identified genetic resources are useful parental materials for sesame breeding programs in Ethiopia and elsewhere.

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Overview of the research findings

Introduction and objectives of the study

Sesame (*Sesamum indicum* L.) is regarded as the queen of oil crops for its unique oil rich seed in human nutrition and commanding high prices in local, regional and international markets. It is widely cultivated in more than 72 countries of tropical and subtropical agro-ecologies globally. The seed oil and derived products have varied uses in the food, feed, and cosmetic industry. Despite the critical roles of this commodity in the agricultural development of various countries in sub-Saharan Africa, including Ethiopia, the mean actual yield of the crop is low (<0.6 ton ha^{-1}) compared with potential yields reaching up to 3 tha^{-1}. The major causes for the low production and productivity of sesame, notably in Ethiopia, are a lack of high-yielding and well adapted varieties; the susceptibility of the currently grown varieties to capsule shattering, biotic and abiotic stresses; lack of modern crop production technologies and poor pre- and post-harvest infrastructure. Thus far, sesame has not received attention from the research and development community compared to other traditional oil crops such as groundnut (*Arachis hypogaea* L.) and sunflower (*Heliantus annuus* L.). Sesame is a traditional and high-value export crop in Ethiopia, but it remains under-researched and underutilized. There is a need for a strong sesame genetic improvement programme to develop and deploy improved varieties with farmer- and market-preferred traits in the country. This study was, therefore, executed with the following major objectives:

i. To document sesame production opportunities and constraints and farmer- and market-preferred varieties and traits in eastern and southwestern Ethiopia as a guide for breeding.
ii. To determine the variance components, heritability and association of seed and oil yield-related traits in Ethiopian sesame genotypes for breeding.
iii. To determine the extent of genetic diversity among 100 sesame genotypes of Ethiopia using phenotypic traits and simple sequence repeat (SSR) markers to select distinct and contrasting genotypes for breeding.
iv. To determine the genetic diversity and relationships among Ethiopia's sesame germplasm collections using seed oil content and fatty acid compositions and diagnostic SSR markers to select genetically unique and promising parental lines for breeding.

Research findings in brief

Appraisal of the sesame production opportunities and constraints, and farmer-preferred varieties and traits, in Eastern and Southwestern Ethiopia

A participatory rural appraisal (PRA) study was conducted in two selected sesame growing regions and four districts in Ethiopia. Data were collected from 160 and 46 sesame farmers through semi-structured questionnaires and focus group discussions, respectively. The main outcomes of this study were as follows:

- Most of the respondent farmers (56.0%) reported cultivating sesame using seeds of unknown varieties often sourced from the informal seed sector.
- The major constraints to sesame production in the study areas included lack of access to improved seeds, low yield potential of the existing varieties, diseases, and low market price, which were reported by 83.0%, 73.8%, 69.4% and 68.8% of respondents, respectively
- True-to-type seed (reported by 36.3% of the respondents), white seed colour (28.8%), and high seed oil content (23.8%) were identified as the most important market-preferred traits of sesame.
- The vital farmer-preferred attributes included reasonable market price, resistance to crop diseases, drought tolerance, resistance to insect pests, higher seed yield, higher thousand-seed weight, higher oil content, white seed colour, early maturity, and good oil qualities such as aroma and taste in order of significance.
- The above key production constraints and market- and farmer-preferred traits are the main drivers of sesame improvement in Ethiopia.

Genetic diversity and association of yield-related traits in sesame

One hundred sesame genotypes were evaluated under field conditions at two locations using a 10 x 10 lattice design with two replications to select better performing genotypes and contrasting parents for breeding. Data were recorded on agronomic traits such as days-to- 50% flowering (DF), days-to-75% maturity (DM), plant height (PH, expressed in cm), internode length (INL) (cm), number of primary branches per plant (NPB), number of secondary branches per plant (NSB), number of capsules per plant (NCPP), number of seeds per capsule (NSPC), stem height to 1st branch (SHB) (cm), distance from lowest branch to 1st capsule (DFLBC)(cm), thousand-seed weight (TSW) (g/1000 seed), seed yield per hectare (SYH) (ton ha^{-1}), oil content (OC) (%), and oil yield per hectare (OYH) (SYH x OC, ton ha^{-1}) for each genotype. The main findings were as follows:

- There existed a higher genotypic coefficient of variation and broad-sense heritability (h^2b) values for NPB, NSB, TSW, SYH and OYH, suggesting the effectiveness of selection for these traits.
- Higher direct effects of OYH and NSPC were recorded affecting SYH, while SYH, NCPP and TSW had a higher direct effect on OYH.
- Genotypes such as Hirhir Kebabo Hairless-9, Setit-3, Orofalc ACC-2, Hirhir Humera Sel-6, Setit-1 and ACC-NS-007(2) were selected for further breeding based on their high seed yield (> 0.73 ton tha^{-1}), oil content (> 53.8%) and oil yield (> 0.40 ton ton ha^{-1}).

Genetic diversity and population structure of Ethiopian sesame (*Sesamum indicum* L.) germplasm assessed through phenotypic traits and simple sequence repeats markers

One hundred sesame entries were field evaluated at two locations in Ethiopia for agro-morphological traits and seed oil content using a 10 × 10 lattice design with two replications. Also, the test genotypes were profiled using 27 polymorphic SSR markers at the Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences. The core findings were as follows:

- Analysis of variance revealed significant ($p \leq 0.05$) entry by environment interaction for plant height, internode length, number of secondary branches, and grain yield.
- Genotypes such as Hirhir Kebabo Hairless-9, Setit-3, Orofalc ACC-2, Hirhir Humera Sel-6, ABX = 2-01-2, and Setit-1 recorded grain yields varying from 0.73 to 1.01 ton ha^{-1}.
- Grain yield had positive and significant associations with oil yield ($p < 0.01$, $r = 0.99$).
- The SSR analysis revealed moderate genetic variation among the assessed genotypes with the mean polymorphic information content of 0.30 and gene diversity of 0.25, needing additional robust and discriminative genetic markers.
- The majority (63%) of the test genotypes showed high membership coefficients to their respective subpopulations, while 37% were admixtures when assessed through structure analysis.
- The test genotypes were differentiated into two and four major heterotic groups based on cluster and population structure analyses, respectively.
- The following genotypes were selected: Hirhir Humera Sel-6, Setit-3, Hirhir Kebabo Hairless Sel-4, Hirhir Nigara 1st Sel-1, Humera-1 and Hirhir Kebabo Early Sel-1 (from cluster II-a), Hirhir kebabo hairless-9, NN-0029(2), NN0068-2 and Bawnji Fiyel Kolet, (from cluster II-b) based on phenotypic and genomic divergence. The selected entries are valuable genetic resources for sesame breeding program in Ethiopia.

Analyses of genetic diversity and population structure of sesame (*Sesamum indicum* L.) genotypes through seed oil and fatty acid compositions and SSR markers

The contents of the seed oil and fatty acids of 100 geniotypes grown under field conditions were determined using the near-infrared reflectance spectrometry. Twenty-seven polymorphic SSR markers were used to assess the genetic profile of the test lines and complement the seed oil and fatty acid data. The main outcomes of this study were as follows:

- The test genotypes showed varied contents of oil that ranged from 44.30 to 55.60%, with a mean of 49.84% followed by oleic acid (36.70 to 48.80%, with a mean of 42.90%) and linoleic acid (36.60 to 47.10%, mean 41.70%).
- The SSR markers resolved the test genotypes into two major clusters, each with two sub-clusters.
- Based on higher oil content and desirable fatty acid compositions and the SSR profiles, the following superior and complementary genotypes were selected: Hirhir Kebabo Hairless Sel-6 (from sub-cluster I-b), Hirhir Humera Sel-8 and NN0058-2 (sub-cluster II-a) and Bawnji Fiyel Kolet (sub-cluster II-b).
- The selected accessions will serve as parents in sesame breeding programs in Ethiopia.

Implications of the findings of the study

Implications of the findings of the study for sesame breeding and genetic improvement emphasising high seed yield, local adaptation, increased oil quantity and quality in Ethiopia

- The PRA study revealed limited access to improved seed, the low yield from cultivating the existing varieties, field diseases and low market price as sesame's most important production constraints. This requires dedicated sesame genetic improvement and marketing strategies in Ethiopia.
- Current and future sesame breeding programs should integrate the key production constraints and market- and farmer-preferred traits to develop and deploy improved varieties that ensure sustainable production, productivity, and adoption of sesame cultivars in Ethiopia.
- There is considerable genetic variability for high seed and oil yields and oil content in the test genotypes to be exploited in sesame breeding.
- Traits such as NPB, NSB, TSW, SYH and OYH, exhibited high genotypic coefficient of variation and h^2b values, suggesting high genetic gains can be achieved through selection.
- The currently used SSR markers were provided complementary data for selecting superior genotypes with high yield and seed oil content. Additional SSR markers should be included in

future studies to establish the heterotic groups for sesame pre-breeding and genetic improvement programmes.

- The current study selected superior and complementary genetic resources based on higher oil content and desirable fatty acid compositions and SSR markers for sesame breeding programs and production in Ethiopia and elsewhere.

Increased seed yield, oil content and quality are the ultimate goals of sesame breeding programs. In the current study, the best-selected genotypes had a mean seed yield of 1.01 tons ha^{-1}, oil content of 55.6%, and oil yield of 0.56 tons ha^{-1} with contrasting genetic profiles. Nevertheless, the average yield recorded in the present genotypes is relatively low compared with the reportedly attainable yield of the crop that can reach up to 3.3 tons ha^{-1}. The low yield performance of the local genotypes is attributable to lack of improved varieties, absence of a formal seed sector, poor agronomic management and extension services, and underdeveloped pre-and post-harvest infrastructures, among others. The local sesame breeding programmes are under-developed and -resourced and focused on agronomic characterisation and mass selection among landrace collections for desirable traits for recommendation and large-scale production. Despite the low yield performance, the local varities are highly preferred by growers, consumers and markets due to their intrinsic seed oil quality characteristics, such as unique aroma and taste. These attributes make the traditional varieties attractive to growers, breeders, and local, regional and international markets. Therefore, current and future sesame genetic improvement programmes should integrate yield and quality promoting traits, local adaptation, capsule shattering resistance, uniform maturity and amenability to machine harvesting, and other industrially essential attributes for multiple utilities. This can be achieved by integrating the conventional breeding methods, mutation breeding, and genetic and genomic techniques such as molecular breeding, genomic-assisted breeding, and genome editing. Additionally, there is a need for vibrant public sector sesame producer cooperatives and the private sector seed industry. These initiatives will strengthen the extension service delivery system and enhances the dissemination and adoption of improved production technologies for sustainable production and economic gains from sesame enterprises.