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**GENETIC STUDIES OF YIELD TRAITS IN SESAME (*Sesamum indicum* L.)
GERMPLASM OF UGANDA**

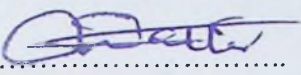
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**A THESIS SUBMITTED TO THE DIRECTORATE OF RESEARCH AND
GRADUATE TRAINING IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF A DOCTOR OF PHILOSOPHY IN PLANT BREEDING
AND BIOTECHNOLOGY OF MAKERERE UNIVERSITY**

DECEMBER 2017

DECLARATION

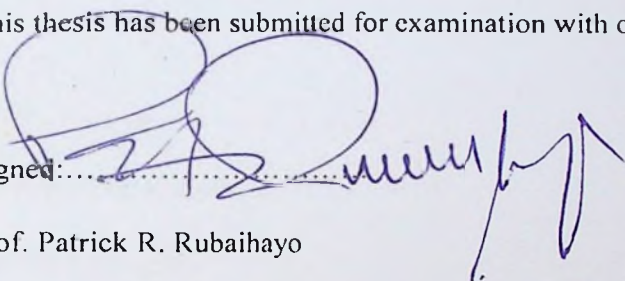
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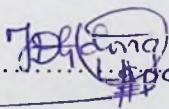
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DECLARATION

I declare that the work presented in this thesis is my own research and it has not been submitted either in whole or in parts for the award of degree at this or any other University.

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DOCTORAL COMMITTEE

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DEDICATION

To my parents, Eresania Dau-Anyanga and Imat Janet Dau for having supported me and encouraged me right from childhood.

ACKNOWLEDGEMENT

"No man is an island unto himself". It is with profound pleasure and much gratitude that I make acknowledgements to those who aided in the success of this thesis. Regional University Forum For Capacity Building in Agriculture (RUFORUM) headed by Prof. Adipala Ekwamu accepted me as one of its students for the PhD programme in plant breeding and biotechnology and provided me with partial sponsorship for my research work. I wish to express my sincere gratitude to my main supervisor, Professor Patrick Rubaihayo for his guidance, suggestions, criticisms and thorough review of both this thesis and the papers that originated from it. His generous help deserves a special mention because without him the thesis would not look what it is now. Dr. Thomas L. Odong who is my second supervisor contributed a lot in editing my chapters and providing necessary comments where improvements were needed. Associate professor Patrick Okori was very instrumental in organizing our training programme in plant breeding and biotechnology. He exposed me to the area of molecular biology, bioinformatics and functional genomics.

Professor George William Otim-Nape, Chairman of the African Innovation Institute (AFRII) seconded me for this PhD training and Drs. Josef Schmidt and Silvia Fluch from Austria fought for the funding of the sesame project that co-funded my PhD programme. Dr. Silvia Fluch also gave me opportunity to work in her laboratory at Austrian Institute of Technology (AIT), Tull, Austria. The project could not be funded if it had not passed through ICRISAT led by Dr. Silim Seyyid. Prof. Johann Vollmann helped partly in the data analysis of my genetic diversity study. Karin Hasel-Hohl supported me a lot in the laboratory work during DNA extraction, PCR analysis and sequencing and Prof Gibson helped in statistical and data analysis

Former Director of NaSARRI, Dr. Beatrice Akello and current Director, Dr. Michael A. Ugen encouraged me in my studies. I am also indebted to the Director General of the National Agricultural Research Organisation (NARO) for having granted me a study leave to complete my study with less work at the Institute. The sesame staff including scientists, technicians and field assistants all supported me both during field work and office activities during my absence. The Trial verification officers (TVOs) of Ngetta ZARDI and Kaberamaido trial verification centres (TVCs) played a key role in supervising my field experiments.

I am indebted to my parents who were very supportive during my training. My wife, Agnes Anyanga and children (Roselyne, Tobias and Joel) accepted my absence as I did my work in Austria and also during the training at Kabanyolo Agricultural Research Institute of Makerere University (MUARIK). Thanks to all my colleagues and well-wishers who encouraged me in one way or the other. Above all, I thank The Almighty God who made everything possible and kept me alive up to the end of the training.

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ABSTRACT

Sesame (*Sesamum indicum* L.) belongs to the family Pedaliaceae in the order Lamiales which contains over 30 species but with *Sesamum indicum* as the only cultivated species. It is the most ancient oilseed crop known to mankind. It is normally called 'Simsim' in Eastern Africa and is a traditional and important oilseed crop in Uganda. It is often referred to by the epithet "the queen of oil seeds" because of its nutritive value, quality and quantity of its oil which is rich in Vitamin E and a significant amount of linoleic acid that can control blood cholesterol levels. Most of the cultivated sesame varieties in Uganda are the local landraces which may be found mixed and are low yielding with the average national yield reported to be about 600 kg/ha. The local landraces which are the main cultivars grown by farmers in Uganda are characterized by poor traits such as few capsules on the main stem zone, setting of the first capsule high up on the main stem from the ground, and wide internode lengths. The overall objective of this study was to contribute to knowledge that will improve on the sesame yield contributing traits through genetic diversity of the germplasm in Uganda. The genetic diversity of Uganda sesame germplasm was assessed from a collection of 115 accessions of sesame landraces and 85 breeding lines including introductions using a set of newly developed microsatellite markers. The results of analysis of molecular variance (AMOVA) for landraces revealed that the majority of the variance occurred among the individuals accounting for 75% of the total variation and only 6% was attributed to differences among the districts, suggesting a high gene flow ($N_m = 4.476$). These results were supported by the PCoA analysis as well as the neighbor joining (NJ) tree which revealed no clustering of the accessions according to their geographic origin. The statistical analysis of 10 agromorphological traits indicated no clear pattern related to the geographic origin. Such a poor grouping was indicative of significant gene flow across geographic domains and could be explained either by a high outcrossing rate and/or through extensive seed trading. Chloroplast-SSR analysis using the marker ccmp2 revealed 3 haplotypes, with haplotype B being predominant and present in 95% of the individuals. The analysis of 9 nuclear SSR regions showed mean H_e values (gene diversity, D) from 0 to 0.373 per line. The gene flow (N_m) among the accessions grouped according to their countries of origin was 1.387 suggesting constant gene flow which was also supported by the observed outcrossing rates of between 3.1% - 62.5%. The AMOVA for breeding lines from different countries indicated that the majority of the variance

was occurring among the individuals accounting for 69% of the total variation and only 11 % of the variation was attributed to differences among the countries of origin. This was also supported by the population structure analysis using the model-based approach revealing 4 groups ($K = 4$), as well as the PCoA and the NJ tree analysis, where neither an origin nor an accession-associated pattern was observed. However, the analysis of 10 agromorphological traits indicated a certain conservation of some morphological traits, reflecting the geographic origin of the analysed lines. For the inheritance and gene action in genotypes for yield traits, the diallel mating design where an 8 x 8 diallel cross mating design with 8 parents and 28F1s showed high GCA effects were recorded for days to flowering, height of first capsule and number of branches, branches per plant and capsule length. Desirable SCA effects were recorded for Sesim2//5181 x Renner 1-3-1-1 for reduced height of first branch, Sesim1 x Sesim2 for number of branches per plant, capsules on branches and yield per plant. High Baker's ratio was recorded for most of the traits except for yield per plant. CGD_{bs} was high for most of the traits except for days to maturity, plant height, capsules on main stem and capsule width. These results suggested that both additive and non-additive gene actions played a greater role on these traits. Sesim 1 had the highest number of desirable traits scored for GCA effects and could be considered as a parent for crossing to produce desirable progeny. Sesim 1 x Sesim 2, Sesim 1 x Ajimo A1-5 and Sesim2//5181 x SPS1438-1-6-4 recorded positive significant SCA effects for yield per plant and therefore they could be recommended for hybrid seed production. The line x tester mating design where nine female local cultivars were used as lines and 5 male introductions were used as testers to produce 45 F1 hybrids which were evaluated together with the 14 parents in three replications revealed that the mean squares across locations had high significant difference for all the traits recorded indicating that locations had effect on those traits. Variances for the males were higher than the variances for the females for all the traits except capsule width suggesting greater variability among the males than the females. Combined general combining ability (GCA) across locations showed that GCA for the lines and testers were significantly positive for a number of traits. Specific combining ability (SCA) was only positively significant for days to flowering, number of capsules on branches, number of capsules on main stem, total number of capsules and capsule length. Baker's ratio was high for most of the characters and medium for plant height, number of capsules on branches, number of capsules on main stem and capsule length. Coefficients of

determination for narrow sense heritability (CGD_{ns}) were high for days to flowering, height of first branch and height of first capsule. Among the lines, Sesim 2, Ajimo A1-5, EM15-1-5, Ajimo A1-6//7029, Local 158//6022 and (Y-1//Local 158)-1-2-1 and among testers 4036-1-10-2 and SPS1438-1-6-4 were desirable for different traits. The results of analysis of genotype x environment interaction to identify stable and high yielding genotypes showed that AMMI analysis of variance for seed yield were highly significant ($P < 0.001$). The combined analysis of variance indicated that season, season x location, genotype and location x genotype had significant ($P < 0.001$) variation. The GGE biplot suggested the existence of only one sesame mega-environment with genotype G9 winning in that mega-environment. Genotypes were categorized into stable and high yielding, stable but poor yielding, unstable but good yielding and unstable and poor yielding.

PUBLICATIONS

This thesis is based on chapters from which the following papers have been published or accepted:

1. Eva M. Sehr, Walter Okello-Anyanga, Karin Hasel-Hohl, Agnes Burg, Stephen Gaubitzer, Patrick R. Rubaihayo, Patrick Okori, Johann Volmann, Paul Gibson, Silvia Fluch. 2015. Assessment of genetic diversity amongst Ugandan sesame (*Sesamum indicum* L.) landraces based on agromorphological traits and genetic markers. Journal of Crop Science and Biotech, 19(1):117-129. Doi No. 10.1007/s12892-015-0105-x.
2. Walter Okello-Anyanga, Karin Hasel-Hohl, Agnes Burg, Stephen Gaubitzer, Patrick R. Rubaihayo, Patrick Okori, Johann Volmann, Paul Gibson, Silvia Fluch and Eva M. Sehr. 2017. Towards the selection of superior sesame lines based on genetic and phenotypic characterization for Uganda. Journal of Agricultural Science; 9(9), 13-35.
3. Walter Okello-Anyanga, Patrick R. Rubaihayo, Paul Gibson and Patrick Okori. 2016. Combining ability and gene action in sesame (*Sesamum indicum* L.) elite genotypes by diallel mating design. Journal of Plant Breeding and Crop Science. 8(11), 250-256. DOI:10.5897/JPBCS2016.0586.
4. Walter Okello-Anyanga, Patrick R. Rubaihayo, Patrick Okori, Paul Gibson and. 2016. Assessment of gene action by line x tester mating design in sesame (*Sesamum indicum* L.) elite genotypes . Journal of Plant Breeding and Genetics, 04(02), 189-202 Doi: 10.5897/AJPS2016.1426.
5. Walter Okello-Anyanga, Patrick R. Rubaihayo, Paul Gibson and Patrick Okori. 2016. Genotype by environment interaction in sesame (*Sesamum indicum* L.) cultivars in Uganda. African Journal of Plant Science. Vol. 10(10), pp. 189-202. DOI:10.5897/AJPS2016.1426.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background

Sesame (*Sesamum indicum* L.) belongs to the family Pedaliaceae in the order lamiales which contains over 30 species but with *Sesamum indicum* as the only cultivated species with chromosomal number ($2n=26$) and an estimated genome size of 369 Mb (Wang *et al.*, 2014). It is the most ancient oilseeds crop known to mankind (Weiss, 1983) and was first domesticated and cultivated on the Indian subcontinent during Harappan and Anatolian eras around 3,500- 3,050 BC (Bedigian and Harlan, 1986). It is often suggested that sesame had its origin in Africa and spread early through West Asia, China and Japan, which became secondary centers of diversity since with the exception of *Sesamum prostratum* Retz, all the wild *Sesamum* species are found in Africa (Purseglove, 1977). The variability and importance of sesame in the economy of several African countries could justify the African continent to be the ultimate centre of origin, however, Bedigian (2004) demonstrated that the crop was first domesticated in India, citing morphological and cytogenic affinities between domesticated sesame and the south Indian native *S. mulayanum* Nair., as well as archeological evidence that showed its cultivation at Harrapa in the Indus Valley between 2250 and 1750 BC.

Sesame is normally called 'Simsim' in Eastern Africa and it is a traditional and important oilseed crop in Uganda (Auckland, 1970). It is also known as *benniseed* (Haruna *et al.* 2012), *gingelly*, *ajonjoli*, and *til* (Ahmed and Ahmed, 2013). It is regarded as a self pollinated crop with 5-60% of out-crossing (Rheenen, 1980). It is one of the most important ancient oilseed crops (Bedigian and Harlan, 1986) and grown throughout the tropical and subtropical regions of the world (Ashri, 1998). Most of the world's sesame growing area is found in India, Myanmar, China, Sudan, Nigeria, with 68% of the total world production (Laurentin and Karlovsky, 2006). It is often referred to by the epithet "the queen of oil seeds" because of its nutritive value, quality and quantity of its oil which is rich in vitamin E and a significant amount of linoleic acid that can control blood cholesterol levels (Vijayarajan *et al.* 2007; Haruna *et al.*, 2012; Salah Balla *et al.* 2014). Despite all these qualities, it is an orphan crop. Little research into sesame has been undertaken as it is not a crop mandated by any international crop research institute.

1.2 World Production of sesame

Sesame is currently grown over a harvested area of 7.8 million hectares worldwide producing more than 3.8 million tons of seed annually throughout the tropical and subtropical regions of the world with Sudan, China mainland, India, and Myanmar being the top producers in 2014, together covering 46 % of the world production with Myanmar as the largest producer of sesame seeds in 2013 (Table 1.1) (FAOSTAT, 2015).

Table 1.1: World sesame production, productivity and area coverage in 2013

No	Countries	Production (tones)	Productivity (Kg/ha)	Area harvested (Ha)
1	Myanmar	890.000	560	1,590.000
2	India	636.000	342	1,860.000
3	China mainland	588.000	1,313	448.000
4	Sudan	562.000	260	2,157,540
5	United Republic of Tanzania	420.000	667	630.000
6	Ethiopia	187.121	661	282.950
7	Uganda	180.000	621	290.000
8	Nigeria	165.000	485	340.000
9	Mozambique	110.000	524	210.000
10	Burkina Faso	95.000	594	160.000

Source: FAOSTAT 2015

On the African continent, Uganda produces (180,000 t) and ranks seventh in sesame production (FAOSTAT, 2013). The world's largest exporter of sesame seeds was India while Japan was the largest importer (FAOSTAT, 2013). Sesame's ability to grow and perform well under moisture stress and low management inputs makes it an important strategic crop among subsistence farmers.

Sesame is cultivated both at smallholder and large commercial scales. It is mainly broadcasted by the smallholder farmers. The seeds are often mixed with sand, soil or ash and then broadcast or drilled by hand in small furrows. Under large scale production, the crop can be planted

mechanically, with the equipment varying from small hand operated seeder units or animal-drawn drills to tractor-operated electronically controlled air seeders.

1.3 Importance of sesame

Sesame seeds have both nutritional and medical value. It is used in sweets such as sesame bars, halva (dessert) and in bakery products or milled to get high-grade edible products (Bedigian, 2004). Sesame seed is also used for decorating bread and cakes as an extra-rich source of protein in many developing countries. The seed has been shown to impart a unique taste and textural features when included with baked products. Seeds are chemically composed of 44-57% oil, 18-25% protein, 13-14% carbohydrates (Borchani *et al.*, 2010). Sesame oil is famous for its stability as a result of its resistance to oxidative rancidity after long exposure to air (Global Agri Systems, 2010) and contains 35% monounsaturated fatty acids and 44% polyunsaturated fatty acids (Hansen, 2011). The sesame oil contains *sesamin* and *sesamolin*. *Sesamolin* is converted to *sesamol* after roasting. The molecular structure of sesamol consists of phenolic and benzodioxide groups. The phenolic group is responsible for antioxidant activities in a number of natural products, while the benzodioxide group is involved in anticancer and antioxidant activities. Since the oil has a long shelf life, it can be blended with less stable vegetable oils to improve their stability and longevity (Chung *et al.*, 2004; Suja *et al.*, 2004). The oil has also medicinal and pharmaceutical value and is used in many health care products. The seeds also are very rich in iron, magnesium, manganese, copper, and calcium and contain vitamin B₁ (thiamine), vitamin E (tocopherol), lignans, phytosterols associated with reduced levels of blood cholesterol, tryptophan and methionine and thus can serve as an important source of proteins in the starch staple diets of subsistence farmers in developing countries. The seed also has ingredients for antiseptics, bactericides, viricides and disinfectants. After oil extraction, the remaining meal which contains high amount of proteins and calcium is used in the animal feed industry. These qualities make sesame a prime commodity in the global market and therefore one with great potential for poverty alleviation, food and nutrition security, household income generation and an important earner of foreign exchange in Uganda.

1.4 Export market of sesame

Sesame derives its value from its many uses both in domestic and export markets (Table 1.2)

Table 1.2: Production in thousand tones and acreage in hactares of oilseed crops in Uganda (2004-2009)

Production	Crop	2004	2005	2006	2007	2008	2009
Metric tonnes	Sesame	125	161	166	168	173	178
	Sunflower	164	173	186	201	217	234
	Groundnut	137	159	154	162	173	185
	Soybeans	158	158	175	176	178	180
Acreage in '000 hactares	Sesame	255	268	276	280	286	292
	Sunflower	769	794	816	835	861	890
	Groundnut	221	225	230	235	244	253
	Soybeans	144	144	145	147	148	150
Export in metric tonnes	Sesame	4,283	7,412	7,568	5,945	14,154	12,109
	Groundnut	1	22	63	101	30	66
	Soybeans	468	574	3,048	5,798	3,250	2,630
Export by value in '000 USS	Sesame	-	4,779	4,547	5,447	15,884	13,369
	Groundnut	-	23	8	148	28	69
	Soybeans	-	126	609	1,331	1,536	1,076

Source: Ministry of Agriculture, Animal Industry and Fisheries (MAAIF), Statistical Abstract 2011.

From Table 1.2, it can be seen that the production of sesame shot up sharply from year 2004 to 2005 and thereafter, the production was steady. Its production was comparable to groundnuts and soybeans. The acreage for sesame production was also high compared to groundnuts and soybeans. Its export and the value of the export were too high to be compared with any of the other oilseed crops recorded. This is an indication that sesame really supports the economy of this country. In some years, although less sesame seed was exported and yet more was produced, this could have been due to less demand by the countries importing it.

1.5 Sesame production in Uganda

Sesame has been cultivated and used in Uganda since 1910 when the crop was first introduced from Kenya (Kisumu) and distributed in Eastern Uganda (Rubaihayo and Okello-Anyanga, 1997). Sesame is predominantly grown in the North and North Eastern parts of the country, although there is a considerable amount grown in Eastern Uganda (Anyanga and Obong, 2001). Sesame is gaining considerable importance in Uganda on account of its economic value partially

for its export potential. Due to variation in rainfall pattern as a result of climate change, the yield is not stable and is still very low averaging about 430 kg/ha (MAAIF and UBOS, 2000).

Between 2002 and 2012, sesame seeds production in Uganda grew by 78% (from 106,000 to 188,740 mt) from an increased area planted by 49% (from 211,000 to 315,000 Ha) (FAOSTAT 2013). With about 93% (93,561 MT) coming from the Northern region in 2008/09 (UBOS, 2008/09) from 158,763 Ha at an average sesame productivity of 0.6Mt/Ha. Sesame is grown in Uganda in two seasons: first season (March-April) and second season (August-September). In good production years, the sesame crop in the second season is significantly larger than the first season due to more area put under the crop as the first season is meant mainly for food crops.

1.5.1 Production constraints

Sesame production may decrease in the foreseeable future due to biotic and abiotic constraints associated with its highly genetic variability especially related to yield structures that act as the basis for sesame-breeding progress (Baydar, 2005).

Most of the cultivated sesame varieties in Uganda are the local land races which may be found mixed or purified by mass selection and are low yielding. No information is available about the desirable and undesirable traits of the varieties being grown. The national yield is reported to be about 600 kg/ha (FAOSTAT, 2009) while Brigham (1985) reported average yields of 1,564 kg/ha in USA.

Uneven ripening of capsules is another major problem in sesame (Wasana Wongyai, 1997) causing shattering. At present, all commercial sesame cultivars in Uganda are indeterminate in growth and thus, yield is lost either because the early produced capsules shatter before harvesting or because late produced capsules are immature at harvest time (Ashri, 1989). Some cultivars take more than four months to mature and are caught in the pronounced drought that follows the growing season.

Pests and diseases also play a major role in reducing sesame yield with the two main pests being sesame webworm (*Antigastra catalaunalis*) and gall midge (*Asphondylia sesami*) (Okidi, 2002). Aphids (*Aphis craccivora*) can also be very destructive when there is serious dry spell. The main

diseases are the angular leaf spot (*Cylindrosporium sesami*), frog eye leaf spot (*Cercospora sesami*) and Fusarium wilt (*Fusarium oxysporium* f.sp. *sesami*) (Engoru and Bashaasha, (2001).

There are a number of abiotic factors that contribute to low yields in Uganda including drought and erratic rainfall, floods in low land and soil fertility (Ssekabembe *et al.*, 2001). With increase in population pressure, soil is getting exhausted because of continuous use without resting or addition of fertilizers. During the seedling stage, weeds suppress the sesame growth and finally affect the production of the crop if not removed.

1.6 Statement of the problem

Productivity of sesame in Uganda is very low due to a number of biotic, abiotic and other related cultural, social and economic factors. The local land races which are the main cultivars grown by farmers in Uganda are characterized by poor traits such as few capsules on the main stem due to short capsule formation zone, setting of the first capsule high up on the main stem from the ground, and wide internode lengths. Some cultivars take more than four months to mature where they are caught in the pronounced drought that follows the rain season. Genetic diversity of the Ugandan sesame germplasm which is important in improvement of the yield characters has not been established. Farmers have continued to grow the local land races because of lack of improved varieties. Only two varieties (Serra and "S") were released in the 1970s and three more (Sesim 1, Sesim 2 and Sesim 3) were released in 2001, 2003 and 2013 respectively but their adoption rate is very low. Farmers face great difficulty in accessing seed in terms of quantity and quality of acceptable seed.

Although sesame research in Uganda started in the early 1960s, no study has been undertaken on the genetics of traits in the available sesame germplasm. It was mainly on maintenance breeding and making a few crosses to get segregating materials. The varieties being grown have not been characterized for the desirable and undesirable traits. Large germplasm collection and characterization need adequate funding and sesame being an orphan crop, lacks this funding. Information on inheritance, heritability, combining ability and gene action though documented elsewhere, is not available for sesame germplasm in Uganda.

The available varieties perform variably across different farmers' conditions and regions and therefore yields fluctuate from area to area. There is lack of knowledge of the nature of combining ability effects that play a role in selection procedure for exploiting heterosis or obtaining desirable progenies. Furthermore, knowledge regarding the nature and magnitude of gene action governing the inheritance of yield and its components is also lacking. Since seed yield is a polygenic character, it is essential to identify yield-contributing traits for selecting high-yielding sesame cultivars.

Pests play a great role in reducing sesame yield with the two main pests that attack sesame being sesame webworm (*Antigastra catalaunalis*) and gall midge (*Asphondylia sesami*) (Okidi, 2002). Aphids (*Aphis craccivora*) can also be very destructive when there is serious dry spelt. Sesame webworm (*Antigastra catalaunalis* Dup.) and gall midge (*Asphondylia sesami* Felt.) were reported to be the most destructive. The field and pest occurrence of *Antigastra catalaunalis* Dup. were recorded to be 100 and 62% respectively while the field and pest occurrence of *Asphondylia sesami* in Uganda were recorded to be 100 and 98.8% respectively (Okidi, 2002). Mean percentage capsule damage due to *Asphondylia sesami* ranged between 29 to 34.3%. The high occurrence of the two pests, the high incidence of *Antigastra catalaunalis* and the high percentage capsule damage by *Asphondylia sesami* suggests that they are of significant importance in sesame production in Uganda. Farmers have a lack of technical knowledge on how to control pests and diseases as the use of chemical insecticides is still limited among sesame growers because of high costs of the insecticides and other associated expenses and lack of the technical know-how in the use of insecticides (Ssekabembe *et al.*, 2001).

The main diseases are the angular leaf spot (*Cylindrosporium sesami*), frog eye leaf spot (*Cercospora sesami*) and Fusarium wilt (*Fusarium oxysporium* f.sp. *sesami*) (Engoru and Bashaasha, 2001). The incidence of disease range between 30.8-84.8%. Crops planted at the onset of rain tend to have the highest incidence because the spores of the fungi require moisture and particular temperature ranges to germinate and infect the leaves (Roger and Windels, 1991). Wilt incidence caused by *Verticillium dahliae* and *F. oxysporium* ranged between 17.1-73.3% (Egonyu *et al.* 2005). Another disease which is of importance is the leaf curl virus disease caused by Nicotiana virus-10 and transmitted by *Bemisia tabaci* (Ashley, 1993) with incidence at the

National Semi-Arid Resources Research Institute, Serere district, ranging between 11-60% and most severely affected crops giving no grain yield at all (Egonyu *et al.* 2005).

1.7 Justification of the proposed research

Crop genetic diversity is the key pillar of agrobiodiversity and is measured by using morphological, biochemical, and molecular markers (Govindaraj *et al.*, 2015).

Morphological traits provide valuable data for cultivar selection to be used in breeding programs (Ansah *et al.*, 2015; Falusi *et al.*, 2015). Molecular markers such as AFLP, ISSR, SSR and RAPD markers have been used for germplasm diversity analysis and the construction of genetic maps (Kumar *et al.*, 2012; Alemu *et al.*, 2013; Zhang *et al.*, 2013). The use of both morphological and molecular markers would elucidate sesame diversity better and in a single system (Sehr *et al.*, 2016).

Molecular markers are more reliable than morphological characteristics for the genetic studies because they are not affected by environmental factors (Ali *et al.*, 2008). Therefore, the ability to distinguish special characteristics and regional varieties requires the molecular level analysis of cultivars.

Knowledge of genetic diversity and relationships among sesame cultivars is essential for selection of parents. Understanding the local genetic diversity by tagging better yield traits using molecular markers could be used to improve the genetic background of the local land races for commercial production.

Understanding combining ability also would assist hybrid combinations and characterizing the nature and magnitude of gene action involved in the expression of quantitative traits. The information on gene action of different effects would also help in the choice of suitable parents for hybridization to be used in the breeding programmes and for selecting the most suitable breeding procedure.

Knowledge of G x E partitioning and interpretation would offer considerable effectiveness in selecting of stable genotypes that interact less with the varying environments.

1.8 Objectives of the study

The overall objective of this study was to contribute to knowledge that will be used to improve on the sesame yield contributing traits through genetic diversity of the germplasm in Uganda. The specific objectives of the study were to:

1. Determine the genetic diversity of Uganda sesame germplasm
2. Determine inheritance and gene action in genotypes for yield traits.
3. Determine the stability and yield performance of the selected elite sesame genotypes in various environments.

1.9 Hypotheses

1. The genetic diversity within the local landraces is equal to the genetic diversity of the breeding lines/introductions currently available in the germplasm.
2. The yield traits in sesame cultivated cultivars in Uganda are controlled by only additive gene action
3. The tested genotypes contain genotypes that combine stability and high yield performance across the selected environments.

CHAPTER TWO

GENERAL LITERATURE REVIEW

2.1 Genetic diversity in sesame

Gangadhara Rao (2001) evaluated seventy two sesame genotypes in a RCBD with two replications where each entry was sown in a single row of 6 m long and at a spacing of 30 x 15 cm. Results showed that there was high heritability and a high genetic advance recorded for plant height, capsules per plant and 1000 seed weight indicating additive gene action in the inheritance of these characters. However, a high heritability and a low genetic advance were observed for days to 50% flowering, days to maturity, number of branches per plant and seed yield per plant indicating non- additive gene action.

Kim *et al.* (2002) evaluated seventy-five accessions of sesame from the germplasm collection of Korea which included 26 Korean cultivars, 17 Korean breeding lines and land races, and 32 introductions from USA, Israel, India, Thailand, Bangladesh, Turkey, Taiwan, Venezuela, Uganda, Pakistan and Japan. One hundred ISSR markers were used to assess their genetic diversity and similarity. The data from the polymorphic bands was used to calculate the genetic distance among the sesame accessions using Nei (1972) and the dissimilarity coefficient matrix was constructed and subjected to unweighted pair-group method for arithmetic averages analysis (UPGMA) to generate the dendrogram using POPGENE 1.32 software (Yeh *et al.* 1997). Results showed that 27 primers did not produce fragments or gave only faint, non-reproductive bands. Fifteen (55%) of the primers had an AT/TA core motif including one that had a TAT motif and five had more than five random nucleotides (n=A,G,C or T). Of the 73 primers that resulted in successful amplification (2-10 products), 43 did not reveal polymorphisms among the lines analysed, while 30 uncovered one to three polymorphic products each. The level of polymorphism ranged from 14.3% to 50% per primer, with a total of 47 polymorphic bands. Among the primers uncovering polymorphic products, 14 generated reliable and reproducible bands. These primers yielded a total of 79 bands of which 29 (33%) were polymorphic. Product sizes ranged from 300 to 3,000 bp. Genetic distances among the accessions ranged from 0 to 0.255. The dendrogram divided the 75 accessions into seven clusters. One of the accessions from

Uganda (EM14) which was officially released as Sesim 1 was in their cluster B with three members.

Kumaresan and Nadarajan (2003) evaluated fifty three sesame genotypes in India in a RCBD with three replications. Ten plants were randomly selected for measurement on seven traits. Results based on D^2 analysis showed that the 53 genotypes were grouped into 19 clusters. Clustering pattern indicated that the genotypes from different areas were grouped into one cluster which could be attributed to free exchange of breeding materials from one place to another.

Young-II Cho *et al.* (2011) developed sixteen polymorphic microsatellite (SSR) markers from an SSR enriched genomic DNA library of sesame (*Sesamum indicum* L) and used them to assess the genetic diversity, phylogenetic relationships and population structure among 150 sesame accessions collected from 22 countries. A total of 121 alleles were detected among the sesame accessions. The number of detected alleles varied from 2-18, with an average of 7.6 alleles per locus. Polymorphism information content values ranged from 0.03 to 0.79, with average of 0.42. These values indicated an excess of heterozygous individuals at 16 loci and an excess of homozygous individuals at three loci. Out of 79 breeding lines, only 13 lines were characterized by private (rare) alleles.

Alemu *et al.* (2013) collected a total of 60 young leaf samples of sesame from sesame growing villages in Ethiopia. Data generated by the three di-nucleotides and one penta-nucleotide ISSR primers were analyzed for 60 individuals of sesame cultivars collected. Shannon's diversity index and analysis of molecular variance (AMOVA) were used to partition the existing genetic variation into within population and among population genetic diversity. The overall analysis of Shannon's diversity index using the four ISSR primers indicated that Bounje showed a higher genetic diversity (0.31) than the remaining five populations. The result also showed that the proportion of genetic variations between sesame populations (0.51) was slightly higher than the within population (0.49). They reported that this might be due to the fact that the same plants for each cultivar and variety were restricted to different districts and farms. High genetic similarity was observed between Bounje and Besh Besh collected from Humera and Metema, respectively with Jaccards similarity coefficient of 0.75. Gojjam Azene and Besh Besh collected from the same area showed the second highest genetic similarity with a 0.70 similarity coefficient. On the

other hand, the least genetic similarity was observed between Hir Hir and Gojjam Azene collected from Armacheho and Humera, respectively, with similarity coefficient of 0.33.

Haiyang Zhang *et al.* (2012) analyzed 24 samples that included four accessions of cultivated sesame (*Sesamum indicum* L., 2n=26), one wild species (*Sesamum radiatum* Schum. & Thonn, 2n=64) and their distant hybrid progeny in RNA-seq experiments. Coefficients of genetic similarity for the 24 cultivated accessions used in this study were calculated using SIMQUAL program of NTSYS-pc Version 2.10 (Rohlf, 2000). A neighbor-joining dendrogram was constructed based on the genetic similarity matrix with the SHAN clustering program of NTSYS-pc using the UPGMA algorithm. PIC ranged from 0.08 to 0.67 and had an average value of 0.34 with primer HS233 giving the maximum PIC value of 0.67. Phylogenetic relationships between the cultivars were assessed by constructing a UPGMA dendrogram using similarity coefficients.

Badri *et al.* (2014) developed 25 microsatellite markers by employing the selective hybridization strategy and 95 mining expressed sequence tags of the NCB1 database. This set of the microsatellite markers was characterized and screened for genetic diversity in an array of 16 sesame germplasms. Of the 120 SSRs (25 genomic SSRs and 95 EST-SSRs), 92 were polymorphic, consisting of 18 SSRs from selective hybridization and 74 from the EST data set. The number of alleles per microsatellite locus ranged from 2 to 5, with an average of 3.11 alleles. The allele size ranged widely (100-510 bp) among the primer pairs. Polymorphic information content estimates ranged from 0.2982 to 0.912. Jaccard's similarity coefficient ranged from 0.21 to 0.82. The potential of the markers was assessed by diversity analysis using the sequential hierarchical agglomerative non-overlapping clustering technique of the unweighted pair group method of arithmetic means on a set of 16 genotypes of sesame, including 2 wild species.

Kun Wu *et al.* (2014) developed and characterized 349 EST-SSR markers from cDNA library and 76 InDel markers from a reduced-representation gDNA library of sesame cultivar Zhongzhi 14. Eighty two important Chinese improved sesame cultivars including 70 white seeded improved cultivars and 12 black seeded improved cultivars, 44 landraces and 4 wild germplasm accessions were used. Only SSR loci of perfect di-, tri-, tetra-, penta-, and hexanucleotide motifs with a minimum of 6, 4, 4, 4, and 4 repeats respectively were evaluated. 349 primer pairs named

as SBM series were designed and synthesized for genetic diversity analysis in sesame. Ninety-seven InDels were identified through comparative Restriction-site Associated DNA (RAD) sequencing of the genomes of 'Zhongzhi 14' and 'Miaoqianzhima', with the GenBank accession numbers KG777470-KG777548. Seventy-nine primer pairs were designed and synthesized for genetic diversity analysis. Then, 88 primer pairs, including 39 EST-SSRs, 13 genomic-SSRs and 36 InDels that amplified reproducible and polymorphic bands were used to genotype 130 sesame cultivars, landraces or wild germplasm. A total of 223 and 102 alleles were detected using SSR and InDel markers, respectively. Allele number per locus for SSR and InDel markers ranged from 2 to 9 and from 2 to 6 (with average number of 4.29 and 2.76), respectively. H_e average was 20.7% and 12.0%, gene diversity average was 0.47 and 0.39, PIC average was 0.40 and 0.32, average minor allele frequency (MAF) was 35.58% and 28.78%, average F_{st} was 0.16 and 0.15, respectively. Genotyping of 130 individuals including white seeded improved cultivars or inbred lines [WIC(L)], white seeded landraces (WLR), black seeded improved cultivars (BIC), black seeded landraces (BLR) and wild germplasm accessions revealed a total of 325 alleles. The average allele number per locus for the five different subsets varied from 2.3034 to 2.9213, with the highest number in wild germplasm accessions. To examine the relatedness among these 130 lines, the genotypic data for 52 SSRs and 36 InDels were analyzed using a model-based approach implemented in STRUCTURE. A neighbor-joining tree and PCA were conducted to examine genetic population structure and genetic clustering of these sesame accessions. The NJ phylogenetic tree based on Nei's genetic distances (1972) displayed a similar clustering pattern or relationship to that of STRUCTURE. The tree had five clear branches with the "mixed" lines distributed in each branch. PCA based on Nei's genetic distances showed, five-cluster distribution pattern, with the mixed subgroups being in the middle of these five defined subgroups.

Abate *et al.* (2015) studied the diversity and population structure of 128 sesame genotypes using ISSR markers and identified highly diverse genotypes of sesame landraces for the purpose of broadening the genetic base of sesame landraces grown in Ethiopia. The 7 ISSR primers in 128 sesame genotypes yielded 96 reproducible amplified bands. The number of amplified bands varied from 7 to 19. The polymorphic information content (PIC) value ranged between 0.26 and 0.76 showing the high informativeness of the selected primers. The overall gene diversity and

Shannon's information index were 0.37 and 0.54 respectively. AMOVA showed lower genetic divergence between populations (6%) than within population (94%) with average F_{st} of 0.061 across populations. They reported that the high intra-population variation could be because of large number of genotypes included and due to high out-crossing nature of sesameranging from 5-65% (Rheenen, 1980; Pathirana, 1994). Clustering and PCoA analyses clustered the genotypes into individual groups where most of the land races were grouped in separate clusters irrespective of their geographic origins, while the cultivars were grouped in one cluster suggesting less variability within the released varieties than the landraces.

2.2 Gene action

Saravanan and Nadarajan (2003) crossed eight sesame genotypes in a half diallel (8 x 8). The eight parents and 28F₁s were evaluated in a RCBD with three replications. Combining ability was analysed using mean values following Model 1, Method II of Griffing (1956). Results showed that variances due to the general combining ability (gca) and the specific combining ability (sca) were significant for all the characters studied. The gca variance was greater than the sca variance for eight traits viz. days to 50%flowering, plant height, number of primary branches per plant, 1000 seed weight, oil content, photosynthetic rate, harvest index and phyllody incidence indicating preponderance of additive gene action for these traits.

Solanki and Gupta (2003) used a diallel set of six genetically diverse sesame parents, evaluated their 15 F₁s along with their 6 parents in a RCBD with 2 replications. Results showed that the mean square for general combining ability and specific combining ability were highly significant for all the characters indicating that both the additive and non-additive type of gene effects were involved in the inheritance of these characters. The magnitude of variance due to general combining ability was higher in days to maturity, branches per plant and capsules per plant suggesting the predominance of additive gene effect for these characters, whereas predominance of nonadditive variance was observed for days to flowering, plant height, capsules bearing plant height and seed yield per plant.

El-Bramawy and Shaban (2007) evaluated six diverse parents of sesame genotypes in a diallel fashion excluding the reciprocals. The six parents and their 15 F₁s were grown in a RCBD with four replications. The analysis of variance revealed that there were highly significant variations

among the 21 genotypes for all the traits investigated indicating considerable genetic diversity among the parents and their respective crosses. The estimates of the components of genetic variance revealed that the additive component (D) was significant for days to maturity, plant height, capsules per plant, and 1000-seed weight. The dominance genetic variances (H_1 and H_2) were significant for all the studied characters except for days to maturity indicating the importance of both additive and non-additive genetic variances in the expression of these characters. Non-additive components of genetic variance was predominant in all the characters, except days to maturity and oil content. The F component was significant and positive for plant height and oil content indicating presence of an excess of dominant alleles. Recessive alleles were of higher frequency for capsules per plant. The value h^2 as a measure of overall dominance effects of heterozygous loci, were significant and positive estimates for days to flowering, plant height, branches per plant, capsules per plant and single plant yield indicated that the mean direction of dominance was positive for these characters. The average degree of dominance ($\sqrt{H_1/D}$) indicated over-dominance for days to 50% flowering, branches/plant, capsules/plant, 1000 seed weight and single plant yield. The gene frequency among the sesame parents that estimates uv and $(H_2/4H_1)$ indicated a symmetrical distribution of genes with positive and negative effects for 1000-seed weight. The quantity of KD/KR was greater than the unity for all the traits, except for fruiting branches per plant (0.72) and capsules per plant (0.82) indicating preponderance of dominant genes in the parents. For fruiting branches/plant and capsules per plant, an excess of recessive genes were evident. The value of K (h^2/H_2) was greater than unity only for capsules on main stem suggesting that one group of genes exhibiting dominance governed that character.

Mishra and Sikarwar (2001) studied five lines used as females and crossed to three testers used as males in a line x tester mating design. The 15 F_1 s and their eight parents were grown in a RCBD in two rows of 5m. Four crosses exhibited significant positive heterobeltiosis for plant height. For number of branches per plant, the majority of crosses showed significant positive heterosis but some were negative. Yader and Mishra (1991) reported also positive heterobeltiosis for number of branches per plant. Seven crosses gave positive significant heterobeltiosis for number of capsules per plant while four crosses gave negative significant values. For number of

seeds per capsule, only three crosses showed significant positive heterobeltiosis. For seed yield, three crosses showed maximum significant positive heterobeltiosis while two gave negative values.

Deepa Sankar and Ananda Kumar (2001) studied eight genotypes crossed as females to four males in a line x tester mating design. The 32 hybrids plus their 12 parents were grown in a RCBD with three replications. Three crosses exhibited a significant negative heterosis, heterobeltiosis and standard heterosis for days to 50% flowering. Three crosses showed significant positive heterosis over mid-parent, better parent and standard parent for plant height. For number of capsules per plant, seven crosses recorded significant positive relative heterosis, heterobeltiosis and standard heterosis.

Solanki and Gupta (2001) studied eleven females and three males used as testers in a line x tester mating design. The 33 F_1 s and their fourteen parents were grown in single row plot with a row length of 2m in a RCBD with two replications. The variances due to males were greater than those of the females for all the characters except for capsules per plant indicating a greater diversity in the males than in the females. The variance due to line x tester interaction was significant for all the characters thereby showing their high specific combining ability. The relative estimates of variance due to sca were higher than those for gca for all the characters except days to maturity and plant height, indicating the predominance of non-additive gene action.

2.3 Genotype by environment interaction and stability of yield

Laurentin and Montila (1999) evaluated eight white-grain sesame genotypes for 3 years and the AMMI model explained 97% of the variation. They found that genotypes and environments located near to the origin were more stable than those far away from the origin which were more responsive. They showed that genotypes and environments that fell into the same sector interacted positively while those that fell into opposite sectors interacted negatively. The ANOVA for the AMMI model showed that the genotypes, environments (years and locations) and $G \times E$ were all highly significant ($P < 0.01$) while the biplot also indicated that the environment conditions in the three years of study were very different. Genotypes interacted positively with some years and negatively with some other years.

John *et al.* (2001) evaluated twenty-five genotypes of sesame grown in three different seasons for seed yield. Three genotypes were recorded to be stable across environments. The mean squares for genotypes, seasons (environments) and G x E interactions were highly significant indicating the existence of a wide range of variation among the genotypes and among the seasons as the performance of genotypes was different over seasons.

Asfaw *et al.* (2009) evaluated eleven soybean genotypes in 4 sites for three cropping seasons (2002-2004) across the soybean production ecology in Ethiopia and reported that AMMI analysis showed that grain yield variation due to environments, genotypes and GEI were highly significant ($P < 0.01$). Environments explained the greater proportion (61.08) of the total yield variation followed by GEI (34.13%) and genotype (4.79%) indicating the necessity for testing soybean varieties at multilocations and over seasons.

Tukamuhabwa *et al.* (2012) evaluated twenty one advanced generation soybean lines together with three standard checks in five sites for three consecutive rainy seasons in 2008A, 2009A and 2009B in Uganda and the AMMI biplot showed that the tested 15 environments (3 seasons x 5 locations) were scattered without any definite grouping with most of the genotypes clustered around the midpoint. The GGE biplot analysis recorded good visual assessment of GG with PCA1 and PCA2 explaining 73.87% of total GE sum of squares. Two sites were identified as highly discriminating for the genotypes tested as evidenced by large environment vectors. Two mega environments with different 'winning' genotypes were identified.

Bo Shim *et al.* (2003) conducted a study to analyze the genotype by environment (G x E) interaction effect on yield in sesame grown in seven different environments by using AMMI analysis. Environments accounted for the largest proportion of the sums of squares (91%) followed by G x E (8%) and genotypes (1%).

Karimizadeh *et al.* (2013) evaluated 18 genetically diverse lentil genotypes over 12 environments for 3 years. The first two principal components of GGE biplot accounted for 49% and 20% respectively of sums of squares of the GE interaction. The combined analysis of variance indicated that year and location were the most important sources affecting yield

variation and these factors accounted for percentages of 50% and 33.3% respectively of the total $G + E + GE$ variation. The GGE biplot suggested the existence of three mega-environments.

Pourded and Moghaddam (2013) evaluated fifteen sunflower inbred lines for four years in six environments. The polygon view of the GGE biplot showed that all test environments were divided into 3 groups. They showed that corner genotypes not in the environments were poor yielders as they were far away from all test locations. They also reported that the genotypes within the polygon were less responsive to location than the corner genotypes.

CHAPTER THREE

GENETIC DIVERSITY AMONG THE LOCAL AND INTRODUCED SESAME GERMPLASM IN UGANDA

3.1 Abstract

Most of the cultivated sesame varieties in Uganda are local landraces which are frequently traded among farmers. Although these traditional landraces are important sources of genetic diversity, knowledge of their genetic diversity is still limited. The objective of this study was to analyse a collection of 115 accessions of Ugandan sesame landraces and 85 breeding lines including introductions using a set of newly developed microsatellite markers. SSR analysis revealed 4 haplotypes, whereby haplotype B was present in 96% of the individuals. The analysis of nSSR markers from 6 non-coding regions revealed a mean PIC value of 0.56 whereas the PIC value of 8 selected EST-derived SSRs was 0.26. Accession-wise, the expected heterozygosity (H_e) varied from 0 to 0.396. Analysis of molecular variance (AMOVA) for landraces revealed that the majority of the variance occurred among the individuals accounting for 75% of the total variation and only 6% was attributed to differences among the districts, suggesting a high rate/degree of gene flow ($N_m = 4.476$). These results were supported by the PCoA analysis as well as the neighbor joining (NJ) tree which revealed no clustering of the accessions according to their geographic origin. Also the statistical analysis of 10 agromorphological traits indicated no clear pattern related to the geographic origin. Such a poor grouping was indicative of significant gene flow across geographic domains and could be explained either by a high out crossing rate and/or through extensive seed trading. Chloroplast-SSR analysis using the marker *ccmp2* revealed 3 haplotypes, with haplotype B being predominant and present in 95% of the individuals. The analysis of 9 nuclear SSR regions showed mean H_e values (gene diversity, D) from 0 to 0.373 per line. The gene flow (N_m) between the accessions grouped according to their countries of origin was 1.387 suggesting constant gene flow which was also supported by the observed out crossing rates of between 3.1%-62.5%. The AMOVA for breeding lines from different countries indicated that the majority of the variance was occurring among the individuals accounting for 69% of the total variation and only 11 % of the variation was attributed to differences among the countries of origin. This was also supported by the population structure analysis using the model-based approach revealing 4 groups ($K = 4$), as well as the PCoA and the NJ tree analysis, where

neither an origin nor an accession-associated pattern was observed. However, the analysis of 10 agromorphological traits indicated a certain conservation of some morphological traits, reflecting the geographic origin of the analysed lines.

Key words: Breeding lines, land races, genetic variation, simple sequence repeats, molecular markers

3.2 Introduction

Sesame breeding strongly relies on the availability of genetic diversity. Thus, understanding existing variation in all its aspects is of utmost importance not only for breeding, but also for conservation and collection activities. Traditionally, studies on genetic diversity were based on morphological and quantitative characters. Several studies have exploited high genetic diversity in populations of sesame by analysing morphological traits only and thereby providing valuable data for cultivar selection for breeding programs (Ansah *et al.*, 2015; Falusi *et al.*, 2015). In order to evaluate existing diversity in landraces and breeding material in depth, traditional approaches need to be combined with modern molecular analyses (Parsaeian *et al.*, 2011; Sehr *et al.*, 2016). Studying diversity on the molecular level needs molecular marker techniques which are highly versatile (Hayward *et al.* 2015).

Both nuclear and organellar SSRs can be used for the evaluation of genetic diversity in plant populations. While nuclear SSRs (nSSRs) are applied for the identification of individual genotypes within a population, chloroplast derived SSRs (cpSSRs) serve for the analysis of the phylogeographic diversity present in a gene pool. An easy accessible source for nSSRs are ESTs (Fluch *et al.*, 2011; Wei *et al.*, 2014). In comparison to SSRs derived from non-coding regions, EST-derived SSRs are located in the coding part of the genome and thereby the polymorphism is somewhat lower and as such variation leads to changes in the coding region and thus in the gene product.

To sustain a level of high productivity and yield, and to face future climatic challenges, sesame breeding will strongly rely on both genetic diversity and genetic purity. For the former, traditional landraces will serve as an important source for breeders; however, the latter is crucial since its loss can compromise yield and other agronomically important traits. For sesame which

has been described as a self-pollinated crop, contradictory outcrossing values have been reported (Andrade *et al.*, 2014; Pathirana, 1994). Elucidating its outcrossing potential is of utmost importance not only for breeding, but also for conservation activities.

Various studies have been undertaken using a wide range/palette of molecular markers in order to assess diversity in sesame with some of the studies based on anonymous dominant markers such as AFLP, ISSR and RAPD for germplasm diversity analysis (Laurentin and Karlovsky, 2006; Sharma *et al.*, 2009) as well as for the construction of genetic maps (Zhang *et al.*, 2013). In recent years, molecular systems have been developed exploiting next generation sequencing techniques (Wang *et al.*, 2014a; Zhang *et al.*, 2012) as well as data mining tools to use EST resources (Yepuri *et al.*, 2013; Bhattacharyya *et al.*, 2014). Despite this increasing amount of studies of the sesame germplasm, knowledge of the genetic diversity of African sesame landraces and wild relatives at the molecular level is scarce and has so far been focused only on Ethiopia (Gebremichael and Parzies, 2011; Alemu *et al.*, 2013; Woldeesenbet *et al.*, 2015) and Nyongesa *et al.* (2013) for Kenya. The study reported in this thesis was intended to unravel the potential of Ugandan sesame genetic diversity in the collection of 115 landraces and 85 breeding lines.

3.3 Materials and Methods

The laboratory work was undertaken at the Austrian Institute of Technology (AIT), Tulln in Austria in 2011. A test set of 17 lines from the National Semi-Arid Resources Research Institute (NaSARRI) (Table 3.1) was used in the preliminary study to select primers that show polymorphism in sesame accessions. Seeds from each accession were germinated and seedlings at 2 to 4 leaf stage used for DNA extraction.

Table 3.1: Genetic material used in the study to select primers

Cultivar	Origin	Characteristics
00001	ICRISAT Project	Green morphology with smooth capsule and stem; susceptible to webworm
EM15-1-5	Local pure line	Green morphology; moderate hairy capsules; tolerant to gall midge; highly branching
OTIS	Foreign/Unknown	Mixed purple and green morphology; long capsules; Low yield
00019	ICRISAT Project	Green morphology; smooth capsule and stem; susceptible to webworm
SESIM2	Released local pure line selection	Purple morphology; short scanty hairs on capsule; smooth stem; drought tolerant; high yield.
00020	ICRISAT Project	Green morphology; smooth capsule and stem; susceptible to webworm
SESIM2//5181-2-2-1	Cross between commercial variety 'Sesim 2' and Thai variety '5181'.	Green morphology; dense hairs on capsule and stem; resistance to gall midge ; high yield
Local 158//6022-1-2-1	Cross between Local 158 from Ethiopia and 6022 from Thailand	Green morphology; dense hairs on capsule and stem; resistance to gall midge
U1-7	Local pure line	Green morphology; smooth capsule and stem; highly branching
Ajimo A1-5	Local pure line	Green morphology; smooth capsule and stem; highly branching
SESIM1	Released Local	Green morphology ; smooth capsule and stem; highly branching
00018	ICRISAT Project	Green morphology ; smooth capsule and stem; susceptible to webworm
Adong 4-4	Local pure line	Green morphology; smooth capsule and stem; highly branching
Local 158	Ethiopia	Green morphology; dense hairs on capsule and stem; 2-4 branches; high yielding; resistance to gall midge; moderately susceptible to wilt
Ajimo A1-6//7029-1	Cross between Local pure line (Ajimo A1-6) and Thai line (7029)	Green morphology; dense hairs on capsule and stem; resistant to gall midge
EM15-3-2	Local pure line	Purple morphology; short scanty hairs on capsule; smooth stem; high yielding; drought tolerant; tolerant to gall midge.
AD-1-1-1	Local pure line	Green morphology; smooth capsule and stem; many branches; high yielding; drought tolerant; tolerant to gall midge and webworm

3.3.1 SSR marker selection and development

The nuclear SSR markers developed for sesame published by Dixit *et al.* (2005) (Table 3.2) were used for pretesting a sample of 17 sesame accessions.

Table 3.2: Nuclear SSR markers used

Locus		Primer sequences (5'-3')	Repeat motif	T _m
GBssr-sa-05	Fwd*	TCA TAT ATA AAA GGA GCC CAA C	(CT)13	54.5
	Rev	GTC ATC GCT TCT CTC TTC TTC		58
GBssr-sa-08	Fwd*	GGA GAA ATT TTC AGA GAG AAA AA	(AG)17	53.3
	Rev	ATT GCT CTG CCT ACA AAT AAA A		52.9
GBssr-sa-09	Fwd*	CCC AAC TCT TCG TCT ATC TC	(CT)18	57.3
	Rev	TAG AGG TAA TTG TGG GGG A		54.4
GBssr-sa-33	Fwd*	TTT TCC TGA ATG GCA TAG TT	(AG)24	51.2
	Rev	GCC CAA TTT GTC TAT CTC CT		55.3
GBssr-sa-184	Fwd*	TCT TGC AAT GGG GAT CAG	(TC)20	53.7
	Rev	CGA ACT ATA GAT AAT CAC TTG GAA		55.8
GBssr-sa-72	Fwd*	GCA GCA GTT CCG TTC TTG	(CT)9	55.9
	Rev	AGT GCT GAA TTT AGT CTG CAT AG		57.1
GBssr-sa-108	Fwd*	CCA CTC AAA ATT TTC ACT AAG AA	(GA)7, (GA)15	53.5
	Rev	TCG TCT TCC TCT CTC CCC		58.2
GBssr-sa-123	Fwd*	GCA AAC ACA TGC ATC CCT	(TC)21, (TC)15	53.7
	Rev	GCC CTG ATG ATA AAG CCA		53.7
GBssr-sa-173	Fwd*	TTT CTT CCT CGT TGC TCG	[(G)CTAGT(G)3(A)2]2	53.7
	Rev	CCT AAC CAA CCA CCC TCC		58.2
GBssr-sa-182	Fwd*	CCA TTG AAA ACT GCA CAC AA	(AT)11, (TC)18, (TG)12	53.2
	Rev	TCC ACA CAC AGA GAG CCC		53.6

Source: Dixit *et al* 2005.

The chloroplast SSR markers developed by Willis *et al.* (2005) were used (Table 3.3) on the sample set of 17 sesame accessions.

Table 3.3: Chloroplast SSR markers used

Locus	Primer sequences (5'-3')		Repeat motif	Tm
ccmp1	Fwd	CAG GTA AAC TTC TCA ACG GA		55.3
	Rev	CCG AAG TCA AAA GAG CGA TT		55.3
ccmp2	Fwd	GAT CCC GGA CGT AAT CCT G		58.9
	Rev	ATC GTA CCG AGG GTT CGA AT		57.3
ccmp3	Fwd	CAG ACC AAA AGC TGA CAT AG		55.3
	Rev	GTT TCA TTC GGC TCC TTT AT		53.2
ccmp4	Fwd	AAT GCT GAA TCG AYG ACC TA		55.3
	Rev	CCA AAA TAT TBG GAG GAC TCT		53.9
ccmp7	Fwd	CAA CAT ATA CCA CTG TCA AG		53.2
	Rev	ACA TCA TTA TTG TAT ACT CTT TC		51.7
NTCP9	Fwd	CTT CCA AGC TAA CGA TGC	T ₁₀	53.7
	Rev	CTG TCC TAT CCA TTA AGA CAA TG		57
NTCP18	Fwd	CTG TCC TTT CCA TGA CCC CTC	T ₁₁	59.7
	Rev	CCA CCT AGC CAA GCC AGA		58.1
NTCP30	Fwd	GAT GGC TCC GTT GCT TTA T	T ₁₃ T ₁₅	54.4
	Rev	TGC CGG AGA GTT CTT AAC AAT A		56.6
NTCP39	Fwd	GTC ACA ATT GGG GTT TTG AAT A	T ₁₃	54.5
	Rev	GAC GAT ACT GTA GGG GAG GTC		61.7
NTCP40	Fwd	TAA TTT GAT TCT TCG TCG C	A ₁₄	50.2
	Rev	GAT GTA GCC AAG TGG ATC A		54.4

Source: David M. Willis *et al.* 2005.

In addition to the already published SSR markers, 23 primer pairs for novel SSR markers were developed based on 3,328 ESTs from NCBI dbEST using the software SciRoKo (Kofler *et al.*, 2007) for screening of SSR regions and the open-source software Primer3 release 1.1.4 (Untergasser *et al.*, 2012) for primer design (Table 3.4).

Table 3.4: List of EST SSRs containing fragments and their SSR motif.

Sequence_Name	Contig_Size	Primer sequences (5'-3')		Repeat Motif	Tm
BU670267	Singleton	Fwd*	GCAACTCCAGTCCCAGTCTC	(GA) ₄	61.4
		Rev	TCCATGGCGTTTGATGAGTA		55.3
BU669001	Singleton	Fwd*	TTGACAATACCGCAATTAGCC	(TG) ₆ , (TC) ₉	55.9
		Rev	CATTGCGTCAGTTGCATTCT		55.3
BU668768	Singleton	Fwd*	TCGCTCTACGTGTTGTTTGC	(TG) ₄	57.3
		Rev	CCAGAACCACCACCTCCTCAT		59.4
BU670685	Singleton	Fwd*	CCCAGCCAAGAAACAAGAAA	(TC) ₉ , (AT) ₄	55.3
		Rev	AACCCCACTAGGCGAAGAAT		57.3
CL45Contig1	6	Fwd*	GACATGGTACGGAGCCAGAT	(CT) ₄	59.4
		Rev	AACCGGTCGAAATCATTCAG		55.3
CL28Contig1	8	Fwd*	CCCAGACTCCATTGCCTTCT	(AT) ₅	59.4
		Rev	TTCTTTGGCTGCTTGGAACT		55.3
CL297Contig1	2	Fwd*	CGAGAAGGAGAACGATTTGG	(AT) ₆	57.3
		Rev	GTAACACTTCCGTCGCCATT		57.3
BU669612	Singleton	Fwd*	GTACAAGAAGCGGCAGCAG	(GC) ₄	58.8
		Rev	CACTCCAACCAACCGATTTT		55.3
CL18Contig1	11	Fwd*	ACACTTTTGGCTCTCGCTGT	(GCA) ₄	57.3
		Rev	GGCCTTGGAGATCACAGAAC		59.4
CL7Contig1	29	Fwd*	GCGTTGTCGTTGGTGTAAA	(CAC) ₄	55.3
		Rev	GTAAACGCCCACAGCGTAAT		57.3
CL370Contig1	2	Fwd*	CCAAGAAACCGCTCACTAGC	(CCA) ₄	59.4
		Rev	CCAGCTCGTACTTCCCATGT		59.4
CL59Contig1	6	Fwd*	GGCATTTGTGACGCAAATAGG	(GCC) ₄	57.3
		Rev	GACGAAAAAGAGCAACAGCAG		57.3
CL93Contig1	5	Fwd*	CAAAGCAAGAGACAAGATGACG	(CCG) ₄	58.4
		Rev	GTGGTGTTTGGACAGTGGTG		59.4

Sequence_Name	Contig_Size	Primer sequences (5'-3')		Repeat Motif	Tm
CL228Contig1	3	Fwd*	CACGCTCAGCATATTTACCG	(GCG) ₄	57.3
		Rev	TCTGTGCAGATTACGGTTTCC		57.9
CL542Contig1	2	Fwd*	TGCGGACTACAACATCCAGA	(AAG) ₄	57.3
		Rev	GAGAGTACCAGCACCGCACT		61.4
CL452Contig1	2	Fwd*	ATACGGCTGCAACTGAGACC	(TCT) ₄	59.4
		Rev	TCCGACAACAAACGAGATCA		55.3
BU670320	Singleton	Fwd*	CCGTTTCGGAGAAAATCAATC	(AAG) ₄	55.3
		Rev	GTTTGGTCCCCCTCTTCTTCC		59.4
CL131Contig1	4	Fwd*	AAACCACATCCCCACATCTC	(TA) ₃ , (CAGA) ₃ , (GA) ₆ , (TA) ₄	57.3
		Rev	TGCACTGCTTACTGCTGAGG		59.4
BU670187	Singleton	Fwd*	CCCACCACITTCACCTTTTCT	(TATG) ₃	57.3
		Rev	CACAGATCCGTAGCTGCAAG		59.4
BU669034	Singleton	Fwd*	CCAATACAAAGCACCCACAA	(TTCT) ₄	55.3
		Rev	GACTCAAAGCACTGCGTGAA		57.3
BU667938	Singleton	Fwd*	TAGGAAGAGGCATGTTACG	(GCAAGC) ₃	57.3
		Rev	CATCTCCACATCTTGCAATCG		57.3
BU669027	Singleton	Fwd*	GGTTCTTTTGCAGGTTGGAA	(GCAATG) ₃	55.3
		Rev	CATCTCCGTTCTGGCAATTT		55.3
CL78Contig1	5	Fwd*	GAGCTCAGTGAGGAGAAAGCA	(TG) ₄	59.8
		Rev	AAGATGAAAGCAGGCAGCTC		57.3

3.3.2 Genotypic Analysis of 17 Test Materials

Two sources of SSR markers were used for this study viz. microsatellite primers which are mostly marking anonymous regions in the genome published by Dixit *et al.*, (2005) (Table 3.2) and electronically available EST sequences (NCBI) with a set of primers by Willis *et al.* (2005) (Table 3.3).

3.3.2.1 DNA extraction

Two capsules per accession were collected and eight seeds from each capsule were germinated in petri dishes. The eight seedlings were each transferred to an eppendorf tube for DNA extraction. The extraction of genomic DNA was performed using the aerial parts of the seedlings following the protocol described in van der Beek *et al.* (1992). After freezing in liquid nitrogen, the seedlings were ground with the help of two steel balls in a Retsch mill and incubated on ice for 10 minutes in a 500 µl of Lefort buffer without CTAB, 100 µl of 10% SDS, 6 µl of proteinase K and 20 µl of 1M dithiothreitol (DTT) were added. The samples were incubated at 60°C in an oven for 4 minutes at 1300 RPM. Then 625 µl of Buffer III (stored at 4°C) was added and shaken on a mixmate at 500 RPM for 1 minute. The samples were thereafter incubated on ice for 10 minutes and again centrifuged at 4600 RPM for 5 minutes at 4°C. Isopropanol was added to the filtrate and centrifuged again. The supernatant was decanted and 70% ice-cold ethanol was added to the centrifugal plate, vortexed and centrifuged before 100 µl of TE8 and 5 µl RNase/well (RNase 10 mg/ml) were added to the pellet to make a solution ready for electrophoresis. A 25 µl PCR mix consisting of 5 µl DNA, 2.5 µl of 10X reaction buffer, 1 mM MgCl₂, 0.25 µl of 20mM dNTPs, 0.4 µl of 4 mM primer forward, 1.2 µl of 4mM primer reverse and 13.4 µl of PCR H₂O were added to form a Mastermix. No enzyme was added at this stage.

3.3.2.2 Assessment of the DNA quality and quantity

The DNA quality was assessed by running the DNA on a 2% agar in a 1xTBE gel. A Mastermix including both forward and reverse primers but without M-13 (Universal primer) was used for checking the quality of the bands and compared with High mass ladder. 2.5 µl of 10X reaction buffer, 1 mM MgCl₂, 0.25 µl of 20mM dNTPs, 0.4 µl of 4 mM primer forward 1.2 µl of 4mM primer reverse and 13.4 µl of PCR H₂O were added to form a Mastermix.

During agarose electrophoresis, 8 µl of DNA was added to 2 µl of blue marker which allows the DNA to sink at the bottom of the well during electrophoresis. The middle range ladder was used to compare the PCR products. Gel electrophoresis was done using 1% agar in a 1xTBE. High mass ladder which has wider fragments was used for comparing the genomic DNA fragments to test the quantity of genomic DNA.

3.3.2.3 Polymerase Chain Reaction

The DNA samples were diluted in order to reduce the load on PCR amplification used to perform polymerase chain reactions. For accurate measurement of size of polymorphic PCR products, the M-13-tail method of Schauke (2000) which involves three primers: a sequence specific forward primer with M13 (-21) tail at its 5' end, a sequence-specific reverse primer and the universal fluorescent-labelled M13 (-21) were used. M13 primer was added to the Mastermix. A 25 µl PCR mix consisting of 5 µl DNA and 20 µl of Mastermix that consisted of 2.5 µl of 10X reaction buffer, 1 mM MgCl₂ (only where Hotstar enzyme was used instead of K-Taq), 0.25 µl of 20mM dNTPs, 0.4 µl of 4 mM primer forward, 1.2 µl of 4mM primer reverse, 0.125 µl of 5 U/µl of either Hotstar enzyme (used for GBssr_sa_108, GBssr_sa_123, GBssr_sa_184 and CL569Contig1) or 0.25 µl of K-Taq (used for GBssr_sa_08, GBssr_sa_72, CL78Contig1, CL93Contig1, CL297Contig1 and ccmp2) and 13.4 µl of PCR H₂O were added to the Mastermix. The forward primer of each SSR was labeled with 6-FAM fluorescent dye from Applied Biosciences. 2 µl of diluted PCR product was added to 8 µl of mixture of Hi-Di (Formamide) which makes the DNA strands to remain separate as single strands and Rox 350 (used for GBssr_sa_08, GBssr_sa_72, GBssr_sa_108, GBssr_sa_123, GBssr_sa_184, CL78Contig1, CL297Contig1 and ccmp2) or Rox 500 (used for CL93Contig1 and CL569Contig1) to make 10 µl for sequencing. After denaturing the DNA products at 95°C for 4 minutes, it was then resolved by capillary electrophoresis on an ABI 3130x1 DNA sequencer. The fragment sizes were automatically collected by GeneMapper 4.0 software and plotted in graphograms.

3.3.2.4 Data collection

The polymorphism information content (PIC) for each SSR marker was calculated according to

the formula: $PIC = 1 - \sum_{i=1}^k P_i^2$, where k is the total number of alleles detected for a locus of a marker and P_i the frequency of the ith allele in the set of 17 lines.

The variability at each locus was measured in terms of the number of alleles, observed heterozygosity (H_O), expected heterozygosity (H_E), genetic distance between each pair of accessions and using the genetic analysis package Arlequin. Major allele frequency (M_{AF}), gene

diversity (GD), polymorphic information content (PIC) were measured by calculating the shared allele frequencies using PowerMarker 3.25 (Liu and Muse, 2005)

3.3.2.5 Genetic Similarity Estimation and Cluster Analysis

Each SSR band was scored as present (1) and absent (0) for the different accessions. Genetic similarity (gs) between two accessions (i, j) was estimated following the formula; $gs_{ij} = 2N_{ij}/(N_i + N_j)$, where N_{ij} is the number of bands present in varieties i and j , N_i (resp. N_j) is the number of bands present in variety i (resp. j) (Nei and Li, 1979). Markers with missing observations for variety i and/or j were not included in the calculation of gs_{ij} . Based on the genetic similarity matrix (denoted gs), Unweighted Pair Group Method of Arithmetic Average (UPGMA) were used to assess pattern of diversity among the sesame accessions. All calculations were performed using the NTSYS-pc version 2.1 software (Rohlf, 2000).

3.3.3 Genotypic analysis of the whole 200 accessions

A total of 115 accessions of sesame landraces (Table 3.5) and 85 introductions (China, Ethiopia, Kenya, Korea, Tanzania, Uganda, USA, and Zimbabwe) (Table 3.6) with details in Appendices 1 and 2 respectively were planted at the National Semi-Arid Resources Research Institute (NaSARRI) in Eastern Uganda in the first rainy season of 2010. The flowers of five plants per accession were selfed and two capsules from the selfed flowers were separately taken per accession from which a number of seeds were germinated in a petri dish. Eight seedlings from each capsule raised in a petri dish were picked for DNA extraction. The extraction of genomic DNA was performed using the aerial parts of the seedlings following the protocol described by van der Beek *et al.* (1992). The remaining selfed flowers of the 200 accessions that formed capsules were harvested and planted during the following first season of 2011 for evaluating agromorphological diversity among the accessions. The experiment was planted in a randomized complete block design with three replications and border rows were planted alongside each replication. The experiment was managed by spraying against pests and diseases and weeded three times during the growing period.

Table 3.5: Number of the sesame landraces collected per district and region used in the evaluation

No	District	Region	No. of land races collected
1	Soroti	Teso	5
2	Kaberamaido	Teso	6
3	Dokolo	Lango	6
4	Lira	Lango	17
5	Amolatar/Awelo	Lango	2
6	Apac	Lango	1
7	Oyam	Lango	12
8	Pader	Acholi	19
9	Kitgum	Acholi	10
10	Gulu	Acholi	16
11	Arua	West Nile	14
12	Nebbi	West Nile	10
13	Masindi	Bunyoro	1

Table 3.6: Number of breeding lines (introductions, pure line crosses and earlier landraces) used in the evaluation

No.	Country of origin	No. of breeding lines
1	Uganda	25
2	Ethiopia	5
3	Korea	1
4	Tanzania	1
5	China	20
6	USA	16
7	ICRISAT/Kenya	14
8	Zimbabwe	1
9	Unknown	1

3.3.3.1 Fragment analysis

PCR amplification was performed as was done for the 17 tested accessions. For studying the landraces and breeding lines, only ten SSR markers consisting of one cpSSR marker (ccmp2) and 9 nuclear SSR markers (CL297, CL569, CL78, CL93, GBssr_sa_08, GBssr_sa_108, GBssr_sa_123, GBssr_sa_184, and GBssr_sa_72) were applied as described (Sehr *et al.*, 2015) via PCR in all 200 accessions. These were the primers that showed good results from work done on the 17 sesame accessions. The resulting PCR products were diluted and mixed with Hi-Di (Formamide) and GeneScan ROX 350/500 dye Size Standard according to the manufacturer's protocols (Life Technologies). The size of the fragments was resolved based on capillary electrophoresis using the ABI 3110 XL Genetic Analyzer. Allele calling was performed using GeneMapper® Software 5 (Applied Biosystems). Non-amplified loci were scored as missing data.

3.3.3.2 Statistical analysis

In order to understand the variability within each group, all the 200 accessions were first analysed together and then they were separated into groups such as the land races, breeding lines, country of origin and re-analysed. Each marker was evaluated for the number of alleles (Na), the observed heterozygosity (Ho), and the expected heterozygosity (He) using GenAlEx 6.5 (Peakall and Smouse, 2012). The polymorphism information content (PIC) was calculated using PowerMarker(Liu and Muse, 2005) according to Anderson *et al.* (1993). To determine the associations between the accessions, statistical parameters (He, Ho, Shannon diversity index [I]) were calculated and an analysis of molecular variance (AMOVA) using 999 permutations was performed using GenAlEx 6.5. Based on resulting pair wise distance matrices, a PCoA plot using GenAlEx 6.5 as well as a neighbor-joining (NJ) tree using MEGA 6 (Tamura *et al.*, 2013) were created to visualize genetic diversity and relationships among the accessions. The resulting tree was further edited using FigTree v1.4.2.

3.3.3.3 Phenotyping and trait statistical analysis

By using the official descriptors for sesame (IPGRI and NBPGR, 2004), the following 10 agromorphological traits were measured in a quantitative approach on the total set of the accessions: days to flowering, days to maturity, plant height, height to first capsule, height to first branch, number of branches per plant, length of capsule zone, number of capsules on main stem, number of capsules on branches, and total capsules. After the whole set of accessions was analysed, accessions belonging to different groups were again analysed differently in order to understand whether there were variability between and within the groups. For an examination of the overall phenotypic diversity across all the 10 traits, individual trait values were standardized using a z-transformation for equal mean and standard deviation. Standardized data were then subjected to principal component analysis and principal component scores were determined for each accession after applying the varimax rotation procedure using SPSS statistical software.

3.3.4. Test of outcrossing rate

In order to assess the rate of out crossing present under natural conditions, using maternal material (DNA from capsule) as well as seed from the same capsule, a half-sib-family analysis was performed. For this, a single farmer’s field was chosen whereby an official released variety

'Sesim 2' was cultivated already for some generations and where off-type individual plants could appear, were collected randomly in 2010. Seeds from each capsule were germinated and DNA was extracted from both, the capsule tissue reflecting the maternal genetics and from seedlings. In this way, genomic DNA of seven different mother plants (A-G) with different capsule locule types and 128 seedlings were investigated (Table 3.7). Each plant was represented by one sampled capsule. From plants A, C, D, and E, 8 seedlings per capsule, and from plants B and F, 32 seedlings per capsule were analysed, totalling to 128 seedling samples. These seeds and the capsules were analysed with 9 markers.

Table 3.7: List of investigated samples for the half-sib family analysis.

Plant	Capsule/type	Seedlings/capsule
A	4-loculed	8
B	4-loculed	32
C	3-loculed	8
D	2-loculed (violet)	8
E	4-loculed (green)	8
F	4-loculed	32
G	4-loculed	32
Total		128

3.4 Results and Discussion

3.4.1 Marker analysis and selection using 17 accessions

3.4.1.1 Nuclear SSR genome analysis

Using the 20 nuclear SSR primers from Dixit *et al.* (2005), 6 proved to be variable in the test set of 17 accessions (Table 3.8).

Table 3.8: Genetic variation in the seventeen sesame accession analysed with six SSR loci using Power Marker v3.25

Marker	Major allele frequency	Genotype No	No of observations	Allele No	He	Ho	PIC
GBssr_sa_08	0.75	6	135	4	0.38	0.12	0.31
GBssr_sa_184	0.44	16	133	8	0.72	0.20	0.68
GBssr_sa_72	0.96	3	135	2	0.07	0.03	0.07
GBssr_sa_108	0.30	21	134	11	0.81	0.18	0.78
GBssr_sa_123	0.43	17	129	11	0.72	0.12	0.69
Sesame09	0.23	15	133	10	0.84	0.05	0.82

Population genetic values were calculated using Powermarker v3.25 software. Different levels of variation with 2 to 11 alleles per locus, and PIC values ranging from 0.07 to 0.82 were observed. Five of these loci were further used on the total dataset. All other five primers except GBssr_sa_72 showed polymorphic information content (PIC) values above 0.3 which is the threshold for primer quality. High polymorphic content indicates that a primer is good for separating variability in alleles among accessions. Oloka *et al* (2015) reported that the number of repeats in the SSR motif had strong correlation with allele number and their polymorphism information content. GBssr_sa_72 had the highest major allele frequency of 0.96 followed by GBssr_sa_08 with 0.75. GBssr_sa_108 and GBssr_sa_123 had the highest number of alleles with 11 alleles followed by Sesame 09 with 10. Most of the markers had high expected heterozygosity except GBssr_sa_72 with 0.07. The same marker also had low PIC of 0.07 compared to other markers suggesting that this locus had low discriminating power and therefore limited in producing good results in differentiating variability in sesame population. Hardy-Weinberg equilibrium in random population should have expected heterozygosity of 0.5. Loci with gene frequency above 0.5 meant that the alleles were more heterozygous and lower than it meant that there were few heterozygous loci from equilibrium. Masudaab *et al*. (2009) reporting in rice showed that a shift from Hardy-Weinberg equilibrium towards zero could be attributed to forces such as inbreeding within groups. Expected heterozygosity is also referred to as gene diversity. Unlike polymorphic information content (PIC), it is estimated from a single locus.

Badri et al. (2014) while screening 16 sesame germplasm with 92 SSRs recorded 2 to 5 alleles per locus and PIC ranged from 0.298 to 0.912.

3.4.1.2 cpDNA Analysis

From the 10 tested chloroplast (cp) primer pairs, 8 worked well in sesame (Table 3.9) and only one of these primers (ccmp2) revealed allelic variation in the set of 17 accessions.

Table 3.9: List of alleles detected with cpDNA primers

Locus	Allele No.	Base pair (bp)
ccmp 1	> 1 peak	
ccmp 2	3	202, 203, 204
ccmp 3	1	119
ccmp 4	1	154
ccmp 5	1	208
NTCP 9	1	276
NTCP 18	1	208
NTCP 30	-	-
NTCP 39	1	160
NTCP 40	1	227

Results showed that almost all the loci indicated only one allele. The locus ccmp2 detected 3 alleles with different base pairs indicating that it was a better primer for separating variability in the accessions. The locus ccmp1 had more than one peak and therefore was difficult to tell the right base pairs. NTCP 30 did not show any allele number and therefore was not useful in the screening of these sesame accessions. With ccmp2 that showed variability in the number of alleles among accessions, three different haplotypes (Haplotype A, B and C) were detected (Table 3.10).

Table 3.10: Chloroplast haplotypes detected with ccmp2 primer for 17 sesame germplasm

Name	Origin	Type of cultivar	Haplotype A	Haplotype B	Haplotype C	Ratio
ICEASE 0001	ICRISAT			X		
EM15-1-5	Local selection (Breeding line)	Pure line	X			
OTIS	Introduction (Unknown country)	Mixed		X		
ICEASE 00019	ICRISAT	Pure line	X		X	7:1
SESIM 2	Local selection released	Pure line		X		
ICEASE 00020	ICRISAT	Pure line		X		
SESIM 2// 5181-2-2-1	A cross between local selection and a Thailand variety (breeding line)	Pure line from a cross		X		
Local 158 x 6022-1-2-1	A cross between local selection and a Thailand variety (breeding line)	Pure line from a cross	X	X		5:3
U1-7	Local selection	Pure line		X		
Ajimo A1-5	Local selection	Pure line		X		
SESIM 1	Released local selection	Pure line		X		
ICEASE 00018	ICRISAT	Pure line		X		
ADONG 4-4	Local selection	Pure line		X		
Local 158	Ethiopia	Pure line		X		
Ajimo A1-6 x 7029-1	A cross between local selection and a Thailand variety (breeding line)	Pure line from a cross	X			
EM15-3-2	Local selection sharing ancestry with Sesim 2	Pure line		X		
AD-1-1-1	Local selection	Pure line		X		

Fourteen out of seventeen accessions belonged to haplotype B. Only 4 accessions had haplotype A. Within these 4 accessions, two of them (EM15-1-15 and Ajimo A1-6//7029-1 showed haplotype A only while ICEASE 00019 showed haplotype A and C in a ratio of 7:1, that is, A haplotype was seven times more common than haplotype C in that accession. It was also observed that, haplotype C was only found in this accession. Local 158//6022-1-2-1 showed haplotypes A and B in a relation of 5:3. Occurrence of different haplotypes in the accessions indicated that there was natural outcrossing in the accessions. This is also an indication that natural outcrossing takes place in sesame crop. The results also indicated that Locus ccmp2 had a discriminatory power in identifying more than one haplotype in the accessions.

3.4.1.3 EST-SSR Analysis

Starting from 3,328 EST-SSRs from NCBI dbEST, 36 primer pairs were generated for subsequent testing with 24 primer pairs revealing good PCR-products, but only 8 loci showed genetic variation in the test set of 17 accessions (Table 3.11).

Table 3.11: Genetic variability in EST-SSR regions used for analysing the seventeen sesame accessions.

Marker	Major allele frequency	Genotype No.	No. of observations	Allele No.	He	Ho	PIC
CL93 Contig 1	0.97	3	130	2	0.05	0.02	0.05
BU668768	0.94	3	135	2	0.12	0.01	0.11
CL297 Contig 1	0.94	3	118	2	0.12	0.03	0.11
BU670685	0.92	3	128	2	0.14	0.03	0.13
CL569 Contig 1	0.78	3	132	2	0.34	0.07	0.28
CL78 Contig 1	0.57	3	127	2	0.49	0.17	0.37
BU670118	0.52	2	126	2	0.50	0.95	0.37
BU670267	0.42	8	119	7	0.73	0.01	0.69

CL93Contig 1 had the highest major allele frequency (0.97) and Bu670267 had the least (0.42). Bu670267 had the highest heterozygosity (H_e) of 0.73 and also the highest PIC (0.69) among the EST-SSR markers selected. Major allele frequency was high ranging from 0.42 in BU670267 to

0.97 in CL93Contig1. Although BU670267 had the lowest major allele frequency, it was recorded in eight genotypes compared to loci which were recorded only in two or three genotypes. It also had seven alleles while other loci had only two alleles. The expected heterozygosity and polymorphic information content (PIC) for this locus were the highest with 0.73 and 0.69 respectively suggesting the superiority of this locus in revealing the genetic diversity in these accessions. It was observed that where major alleles were high, the expected heterozygosities (H_e), observed heterozygosities (H_o) and PIC were low suggesting that where major alleles were high, less information and genetic diversity was provided in the accessions.

The results of detected alleles in the 17 accessions ranged from 1 to 5 per locus (Table 3.12) clearly indicating that this set of 14 loci (6 nuclear SSRs and 8 EST SSRs) were discriminatory and revealed a high level of genetic variability throughout the accessions tested. There were more alleles revealed from nuclear markers than genic markers in this study indicating greater diversity in the materials used. When comparing the allelic frequency of gene related versus 'anonymous markers' it was observed that the gene related SSRs showed reduced variability and less alleles per locus as compared to the other SSRs. This can be attributed to the fact that the selection pressure on coding regions is a lot higher than on non coding regions indicating that mutations in non coding regions can be tolerated much more easily and thus the mutation rate in these regions was higher). The number of alleles present in the different lines, in some loci up to 5 alleles were detected showing that the discriminating power of some of these loci in identifying the accessions that have the potential to be of major interest in a breeding program.

Table 3.12: Alleles detected per locus by 14 loci within each of the 17 accessions

Cultivar	BU668768	BU670267	BU670685	CL297 Contig1	CL93 Contig1	GBsrr_sa_08	GBsrr_sa_184	GBsrr_sa_72	GBsrr_sa_108	GBsrr_sa_123	BU670118	Sesame 09	CL569 Contig1	CL78 Contig1
ICEASE 0001	1	2	2	1	1	1	3	1	4	2	2	2	1	1
EM15-1-5	2	3	1	1	1	2	3	1	4	3	4	2	2	2
OTIS	1	3	1	1	1	2	4	1	2	5	5	2	2	2
ICEASE 00019	2	3	2	2	2	1	3	2	3	1	5	2	1	2
SESIM 2	1	3	1	1	1	2	2	1	2	2	5	2	2	2
ICEASE 00020	1	4	1	2	2	1	4	2	5	4	3	2	1	2
(SESIM 2 x 5181)-2-2-1	1	2	1	1	1	1	1	1	2	1	2	2	1	2
(Local 158 x 6022-1)-2-1	2	4	1	1	1	2	3	1	3	3	4	2	2	2
UI-7	1	1	1	1	1	1	2	1	2	1	4	2	1	2
Ajimo A1-5	1	2	1	1	1	2	2	1	4	3	4	2	2	2
SESIM 1	1	2	1	1	1	3	3	1	4	2	3	2	1	2
ICEASE 00018	1	2	2	1	1	1	3	1	2	2	1	2	1	1
ADONG 4-4	1	2	1	1	1	2	3	1	5	3	3	2	1	2
Local 158	1	3	1	1	1	2	2	1	3	2	3	2	1	2
Ajimo A1-6 x 7029-1	1	3	1	1	1	2	3	1	3	2	2	2	2	2
EM15-3-2	1	2	1	1	1	3	3	1	5	2	4	2	2	2
AD-1-1-1	1	2	1	1	1	1	2	1	2	3	1	2	2	2

The results of the genetic relationships among the 17 accessions are presented in a dendrogram based on Nei's genetic distance (Fig.3.1).

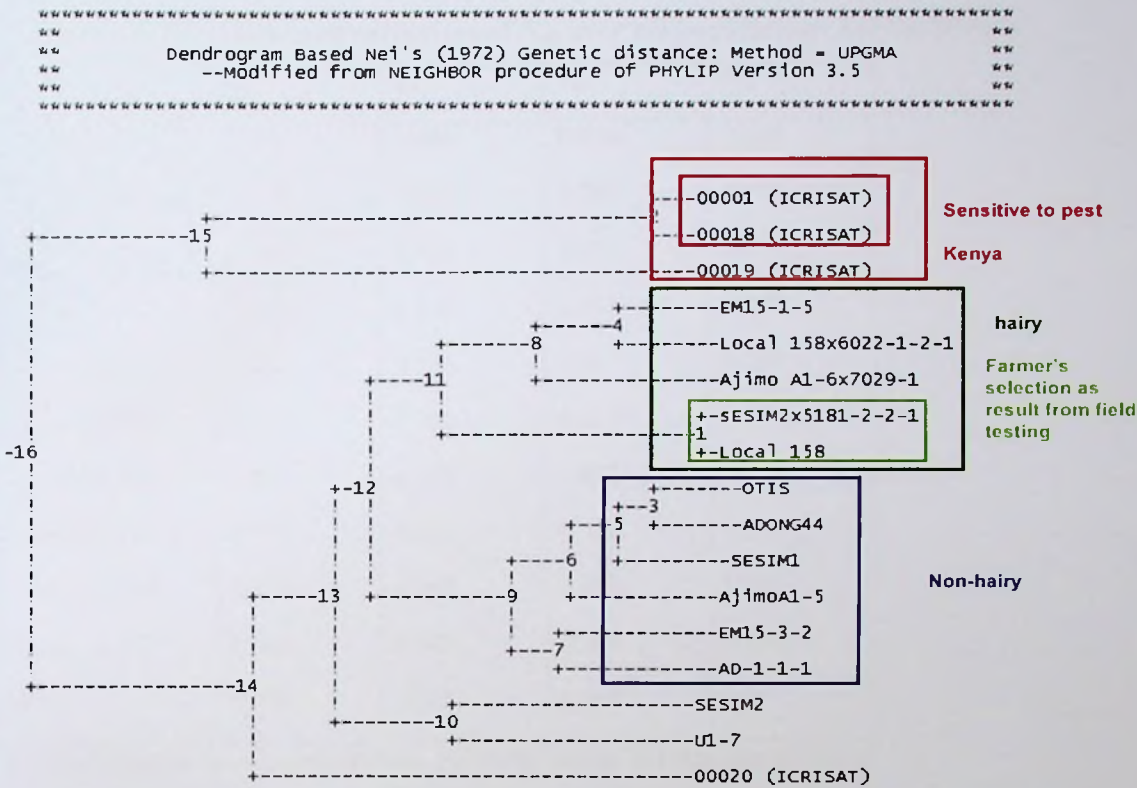


Figure 3.1: Dendrogram for the 17 analyzed accessions showing genetic distance

The results revealed that the 17 germplasm lines fell into four different clusters, where three accessions from ICRISAT formed a clade on their own. Another observation was that accessions which shared phenotypic similar trait of hairiness (on the capsules, flowers and stem) fell together in a cluster, and two accessions which were rated as best performing in the farmer's field trials (Local 158 and (Sesim2//5181)-2-2-1) show very close genetic relation. Due to their hairiness, they tended to show resistance to gall midge pest (*Asphondylia sesami* L.) that causes abortion and galled capsules in sesame and therefore less damage (Ogwal *et al.* 2000).

3.4.2 Population structure analysis

3.4.2.1 Population structure analysis of the whole 200 accessions

Genetic population structure of the 200 Uganda sesame germplasm based on nine SSR markers (5 nuclear SSRs and 4 EST-SSRs) markers are presented in Table 3.13.

Table 3.13: F-statistic and estimates of N_m over all populations for each locus

Locus	F_{is}	F_{it}	F_{st}	N_m
ccmp2	1.000	1.000	0.868	0.038
CL297Contig1	0.797	0.956	0.781	0.070
CL569Contig1	0.493	0.735	0.477	0.274
CL78Contig1	0.585	0.818	0.562	0.195
CL93Contig1	0.227	0.576	0.451	0.304
GBssr_sa_08	0.487	0.762	0.536	0.217
GBssr_sa_108	0.505	0.748	0.492	0.258
GBssr_sa_123	0.478	0.753	0.526	0.225
GBssr_sa_184	0.523	0.747	0.470	0.282
GBssr_sa_72	0.048	0.803	0.793	0.065
SE	0.083	0.038	0.049	0.031

F_{is} =individuals in sub-population, F_{it} =individuals in total population.

F_{st} =sub-population in total population, N_m =gene flow or gene migration.

ccmp2 had high F_{is} and F_{it} at unity and F_{st} both at 0.868 but had the lowest N_m . The mean values of F_{is} , F_{it} , F_{st} and N_m were 0.514, 0.790, 0.596 and 0.031 respectively. All the loci had high F_{it} and F_{st} above 0.5 except CL569Contig1, CL93Contig1, GBssr_sa_108 and GBssr_sa_184. N_m was generally low for all the loci. The highest was CL93Contig1 with 0.304 and the lowest was ccmp2 with 0.038. The fixation index (F_{st}) values were generally high (0.451 to 0.868) indicating that high levels of genetic differentiation were present in the sesame populations sampled. " F_{st} " is the most appropriate index of gene-frequency differentiation if and only if the genetic diversity is low (Nagylaki, 1998). Fixation index is a measure of population

differentiation arising from their genetic structure. The value ranged from 0-1. Zero implies complete panmixis, that is, the populations are interbreeding freely. A value of one means that the populations do not share any genetic diversity and that all the variation arising from them can be explained by their population structure (Holsinger *et al.*, 2009).

3.4.2.2. Population structure analysis of 115 local landraces

Accessions 90, 121, 160, and 189 of the landraces were excluded from the molecular analysis due to failure of their PCR amplification and 9 loci were scored for the remaining landraces which were sampled in 13 districts in Uganda. Heterozygosity and polymorphism were calculated for each locus (Table 3.14) and for each accession separately. The mean H_o values per locus were very low (0 – 0.184), mean H_e values ranged from very low to fairly high (0.004 – 0.683). The low H_e values in this study can be partly explained by the fact that the microsatellites were developed from ESTs, which are generally less variable than those from non-coding regions of the genome. The calculated Shannon diversity index (I) ranged from 0 to 0.624 (grand mean = 0.298), indicating various depths of genetic diversity within the accessions. The mean H_e values (or gene diversity, D) varied from 0 to 0.396 (grand mean = 0.084), and the H_o values range from 0 to 0.299 (grand mean = 0.194).

Table 3.14: Heterozygosity, F-statistics and polymorphism presented as mean values per locus for the landraces.

Parameters	Nuclear SSR and EST-SSR markers								
	CL297	CL569	CL78	CL93	GBssr_sa_08	GBssr_sa_108	GBssr_sa_123	GBssr_sa_184	GBssr_sa_72
	Contig1	Contig1	Contig1	Contig1					
N	141.769	140.154	140.846	139.538	140.385	141.615	143.231	143.385	141.769
Na	1.154	2.000	1.923	1.077	3.538	6.308	5.538	5.077	1.462
Ne	1.010	1.580	1.668	1.005	2.025	3.414	3.764	3.037	1.014
I	0.021	0.519	0.546	0.011	0.814	1.371	1.357	1.221	0.031
H_o	0.003	0.087	0.075	0.000	0.104	0.184	0.127	0.137	0.003
H_e	0.009	0.348	0.375	0.005	0.465	0.679	0.683	0.646	0.013

Sample size (N), number of alleles (Na), number of effective alleles (Ne), and Shannon diversity index (I), observed heterozygosity (H_o), and expected heterozygosity (H_e) calculated as mean values using GenAlEx.

After grouping the accessions into 13 Ugandan districts (Table 3.15), the degree of variation within a specific district, measured by the Shannon's diversity index (H'), was in general very high (0.420 – 0.809), except for accessions collected in Masindi and Apac. This could have been due to the fact that only 1 accession each was collected from these districts. In all cases, H_e was greater than H_o . Five districts were characterized by private alleles: Dokolo (GBssr_sa_123: allele 1), Gulu (CL569Contig1: allele 3; CL93Contig1: allele 2; GBssr_sa_123: allele 15), Lira (GBssr_sa_08: allele 1; GBssr_sa_184: allele 10), Oyam (GBssr_sa_184: allele 3), and Pader (GBssr_sa_108: allele 11). The overall estimate of gene flow (N_m) among populations of Ugandan sesame accessions considering the districts as independent population was rather high with 4.476, which might be due to a high outcrossing rate, which has been described for sesame to be between 1 and 68 % (Pathirana, 1994) and/or because of anthropogenic effects such as extensive seed trading.

Table 3.15: Characteristics of heterozygosity of farm collected and market samples.

No of accessions per district and collection site type															
Parameters	Apac	Arua	Awelo	Dokolo	Gulu	Kberamaido	Kitgum	Lira	Masindi	Nebbi	Oyam	Pader	Soroti	Farm	Market
	1	15	2	5	15	3	10	18	1	9	13	21	4	102	15
Na	1.778	3.222	2.333	3.222	3.778	2.556	3.111	4.222	1.667	3.444	4.222	4.333	2.667	6.444	3.889
Ne	1.608	2.331	1.951	1.943	2.027	1.582	2.060	2.358	1.615	2.429	2.466	2.270	2.106	2.421	2.212
I	0.429	0.756	0.597	0.655	0.709	0.499	0.660	0.785	0.420	0.765	0.809	0.761	0.665	0.830	0.744
No. priv. alleles	0.000	0.000	0.000	0.111	0.333	0.000	0.000	0.222	0.000	0.000	0.111	0.111	0.000	2.556	0.000
He	0.275	0.398	0.341	0.343	0.368	0.312	0.351	0.416	0.288	0.389	0.411	0.395	0.367	0.407	0.382
Ho	0.111	0.073	0.092	0.068	0.064	0.072	0.080	0.113	0.063	0.078	0.105	0.085	0.036	0.085	0.080

Na=Number of different alleles, Ne=Number of effective alleles, I=Shannon index, He= expected heterozygosity,
Ho= observed heterozygosity

3.4.2.3: Population structure analysis of 85 breeding lines

Nine loci were scored for 85 sesame breeding lines and stabilized crosses of Uganda based collection. Heterozygosity and polymorphism were calculated for each locus (Table 3.16) and for each accession separately.

The mean H_o values per locus were very low (0.00 – 0.31), mean H_e values were in the range of 0.03 – 0.83. Some of the low H_e values in this study could be partly explained by the fact that the microsatellites were developed from ESTs, which are generally less variable than those from non-coding regions of the genome. This is in line with previous studies, where a gene diversity (H_e , D) between 0 and 0.440 was described in African sesame lines (Gebremichael and Parzies, 2011; Nyongesa *et al.*, 2013; Sehr *et al.*, 2016).

Table 3.16: Heterozygosity, F-statistics and polymorphism presented as mean values per locus for breeding lines.

Parameters	Nuclear markers											
	CL297 Contig1	CL569 Contig1	CL78 Contig1	CL93 Contig1	GBssr_sa_08	GBssr_sa_108	GBssr_sa_123	GBssr_sa_184	GBssr_sa_72			
N	657	647	656	649	653	645	658	660	625			
Na	2	4	2	3	7	15	14	11	3			
Ne	1.03	1.86	1.98	1.06	1.88	4.86	5.79	4.26	1.09			
Ho	0.00	0.16	0.15	0.03	0.17	0.30	0.31	0.27	0.04			
He	0.03	0.46	0.50	0.06	0.47	0.79	0.83	0.77	0.00			
F	0.91	0.65	0.69	0.44	0.64	0.62	0.63	0.65	0.56			

Sample size (N), number of alleles (Na), number of effective alleles (Ne), Shannon diversity index (I), observed heterozygosity (Ho), expected heterozygosity (He), and the fixation index (F) calculated as mean values using GenAlEx.

After grouping the accessions according to their country of origin, the degree of variation within a specific country of origin, measured by the Shannon's diversity index (*I*), ranged from a low value of 0.237 up to 0.936 (Table 3.17).

Table 3.17: Genetic diversity of breeding lines measured across the countries of origin.

Parameters	Country of origin and no. of accessions per country							
	China	Ethiopia	Kenya	Korea	Tanzania	Uganda	USA	Zimbabwe
	20	5	14	1	1	25	16	1
Na	4.000	2.667	4.778	1.444	1.778	4.111	4.778	1.889
Ne	2.492	1.722	2.590	1.268	1.461	2.294	2.394	1.730
I	0.795	0.571	0.936	0.237	0.377	0.769	0.806	0.493
No. priv. alleles	0.222	0.000	0.778	0.000	0.000	0.111	0.333	0.000
He	0.403	0.330	0.462	0.158	0.253	0.394	0.415	0.322
Ho	0.120	0.072	0.069	0.132	0.111	0.111	0.120	0.118

The results showed that the number of alleles ranged from 1.444 – 4.778, with the highest allelic richness seen in the lines from Kenya and the USA. Lines from Korea, Tanzania, and Zimbabwe showed very few numbers of alleles but also these countries of origin comprised only one accession each. Young-IL Cho *et al.* (2011) detected 121 alleles while evaluating 150 sesame accessions from 22 countries. The number of countries was many enough to detect such number of alleles compared to this study. Four out of the 8 countries of origin were characterized by private alleles: China, Kenya, Uganda and USA. The overall estimate of gene flow (*N_m*) between the countries of origin was 1.387, which is less than what is seen in Ugandan landraces where a gene flow of 4.476 was detected between different districts (Sehr *et al.*, 2016), but the value still exceeds 1, indicating a constant gene flow between accessions from different countries of origin. This amount of gene flow, which is naturally due to either seed dispersal and/or via cross-pollination, can be also explained by anthropogenic effects such as extensive seed trading and deliberate introductions by plant breeders (Edossa *et al.* 2007).

3.4.3. Analysis of Molecular variances (AMOVA)

An AMOVA analysis was used to evaluate the diversity components within and among the accessions, which had been grouped into the respective districts. The majority of the variance was occurring among the individuals accounting for 76 % of the total variation, and only 5 % of the variation was attributed to differences among the districts (Table 3.18).

Table 3.18: Analysis of molecular variance (AMOVA) based on 9 SSR markers for sesame landraces grouped into 13 districts.

Source of variation	d.f.	Sum of squares	Mean squares	Percentage variation
Among districts	12	380.533	31.711	5%
Among individuals	1,859	6,180.372	3.325	76%
Within individuals	1,872	695.000	0.371	19%
Total	3,743	7,255.905		100%

Similar results where differences among geographical regions were represented by only 5 % of the total variation in sesame accessions were described by Laurentin and Karlovsky (2006) and Woldesenbet *et al.* (2015). When analyzing the molecular variance among the accessions bought at the market and the accessions collected at farmer sites, only 1 % of the total variation was attributed to differences among these two sources, whereas 80 % of the total variation occurred among the accessions. This very low among group variance could be explained with high gene flow represented by seed movement most probably through human involvement (Edossa *et al.* 2007). Abate *et al.* (2015) while studying diversity and population structure of 128 sesame land races in Ethiopia using ISSR primers recorded AMOVA data to show 6% of genetic divergence between populations and 94% within population which is similar to this study.

An AMOVA analysis was used to evaluate the diversity components within and among the accessions, which had been grouped into the respective countries of origin. The majority of the variance occurring among the individuals accounted for 56 % of the total variation, and 7 % of the variation was attributed to differences among the countries of origin (Table 3.19).

Table 3.19: AMOVA based on 9 SSR markers and 83 sesame breeding lines grouped into 8 countries of origin.

Source of variation	d.f.	Sum of squares	Mean sum of squares	Percentage of variation
Among countries of origin	7	168.13	24.02	7 %
Among individuals	658	1994.58	3.03	56 %
Within individuals	666	492.43	0.74	36 %
Total	1331	2655.14		100 %

Woldesenbet *et al.* (2015) while using ISSRs to analyse genetic diversity among the Ethiopian landraces and exotics revealed that AMOVA without grouping recorded higher percentage of 94.09% variation attributed to the within population variation while 5.90% due to the among population variation. When AMOVA analysis was grouped, considering the Ethiopian accessions as one group and the exotic ones as another, it showed 5.88% among population variation, 94.09% within group variation and 0.026% among group variation.

Principal coordinate analysis of the genetic diversity for all the 200 accessions was done and the results are presented in Figure 3.2.

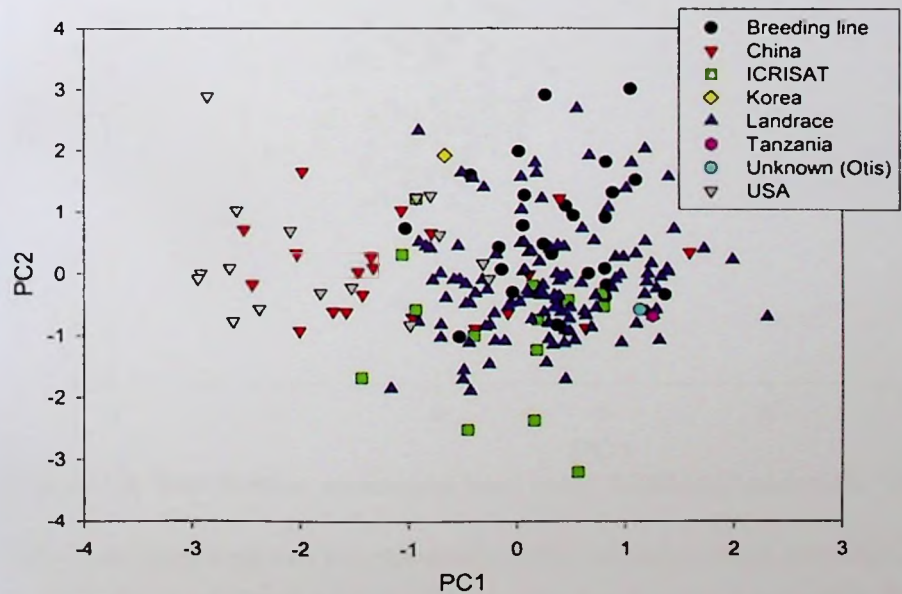


Figure 3.2: Genotypic diversity of sesame genotypes in Uganda germplasm.

USA accessions are located on the left hand of the graph and next to the the USA accessions are China materials. The ICRISAT materials are mainly found below the lower part of the graph. A few breeding lines and landraces were also found among these USA, China and ICRISAT materials. This could be due to outcrossing and selected as purelines after recombinations of genes. The majority of land races and breeding lines are found on the right hand of the graph. For the breeding lines, these are mainly crosses among the selected local landraces and introductions thus leading to segregation and recombination of genes. Since sesame is a self-pollinating crop with some percentage of outcrossing (5-60%) (Rheenen, 1980) and the germplasm maintained is not by controlled self pollination, it is possible that there has been outcrossing resulting into segregation and recombination of genes.

Distribution patterns of land races, ICRISAT materials, USA and China is presented in Figure 3.3.

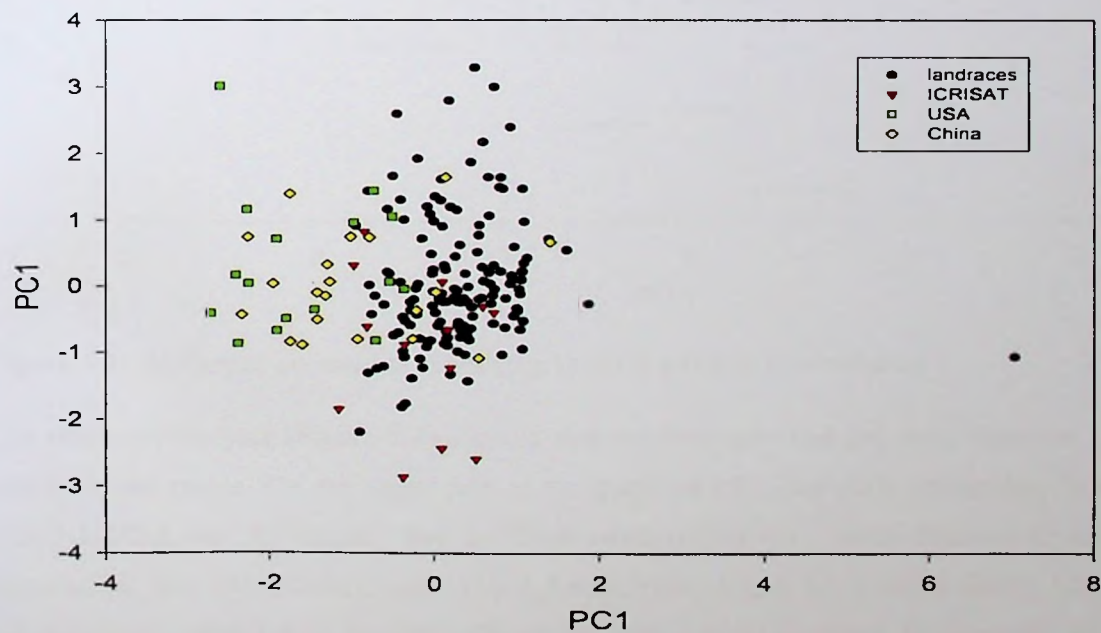


Figure 3.3: Distribution patterns of land races, ICRISAT materials, USA and China

USA and China materials are clustered together on the extreme left while landraces are clustered in the centre and ICRISAT accessions in the lower part of the graph. There are some distinct patterns among the origin of the accessions. The ICRISAT materials were observed also distinct

on the lower part of the graph. The accession ‘Oyam hairy’ which has no known origin but considered now as a landrace since it is being grown locally by some few farmers in Oyam district is seen to be quite distinct at the extreme right hand side of the graph. The cultivar Oyam matures in 130 days compared to all other accessions that mature within 100 days. Most landraces were mainly observed to be clustered in the middle of the graph.

The principal coordinates of all the 200 accessions are presented in Figure 3.4.

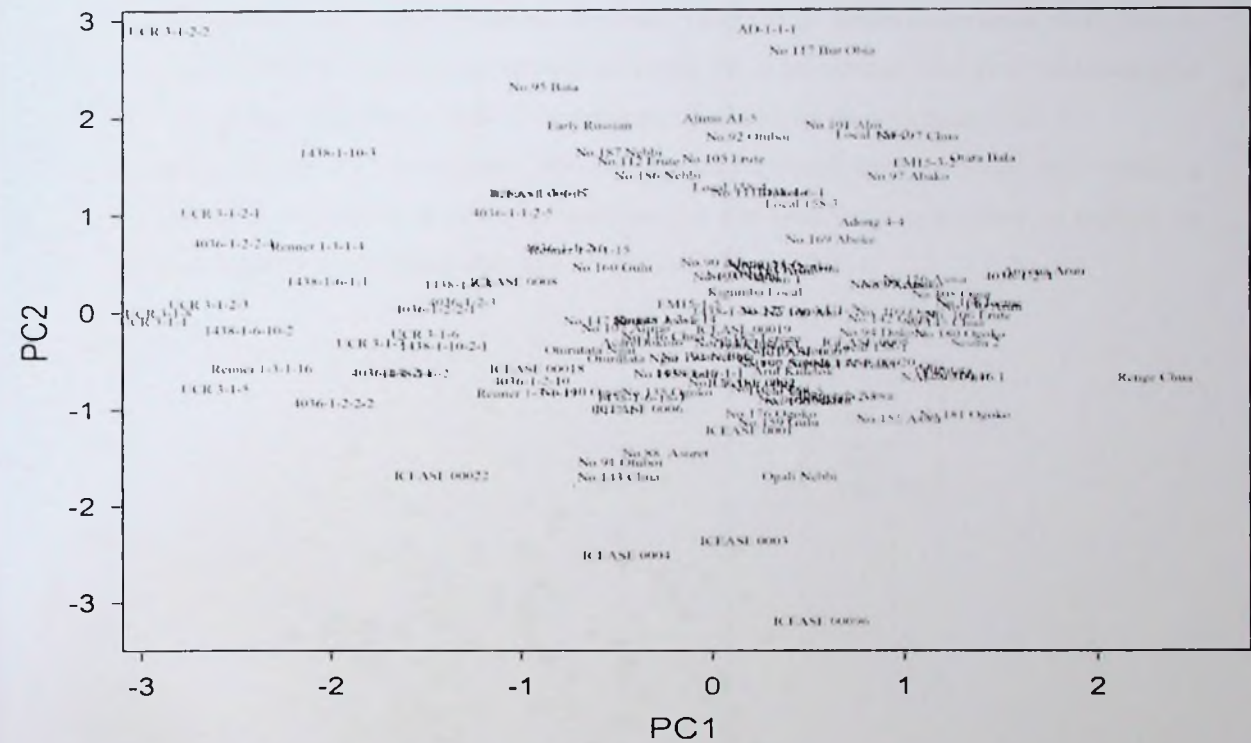


Figure 3.4: Different accessions according to their pattern of similarity

The results of analysis (Figure 3.4) showed that the landraces and breeding lines are clustered mainly in the centre. On the upper part of the graph of PC2, the main accessions found were UCR3-1-2-2-2, No. 95 (Bata), No. 117(Bur- obia), AD-1-1-1, Early Russian (from Korea) followed by No. 186 (Nebbi), Local 158-2, Local158-4, Ajimo A1-5, Otara (Bala), EM-15-3-2, No.101(Aloi), 1438-1-6-1, No.197 (Chua), Adong 4-4. This could be grouped as similar accessions with similar genetic constitution. On the extreme left of the graph of PC2 were UCR3-1-8, UCR3-1-4, UCR3-1-2-3, UCR3-1-5, 1438-1-6-10-2, 4036-1-2-2-4, Renner 1-3-1-4.

These are cultivars from USA and China. They have the characteristics of earliness, lanceolate leaves, shorter internode length and multicapsules. On the lower part of the graph ICEASE 00096 was the most extreme towards PC1 followed by ICEASE 00004 and ICEASE 00003 respectively. Others observed were the accessions ICEASE 00002, No. 143 Chua, No. 91 Otuboi, Ogali, No. 110 Ogur, and No. 86 Asuret. In order to make crosses that could produce good segregating lines and recombinations, it would be good to select accessions according to these groupings. The USA and China accessions could be in one group, the local and breeding lines in another group while the ICRISAT accessions would be on their own as well.

After analysing all the 200 accessions, they were again divided into landraces and breeding lines. The principal coordinate graph after analysis for the land races according to regions of tribal setting in Uganda where those districts are located (Figure 3.5).

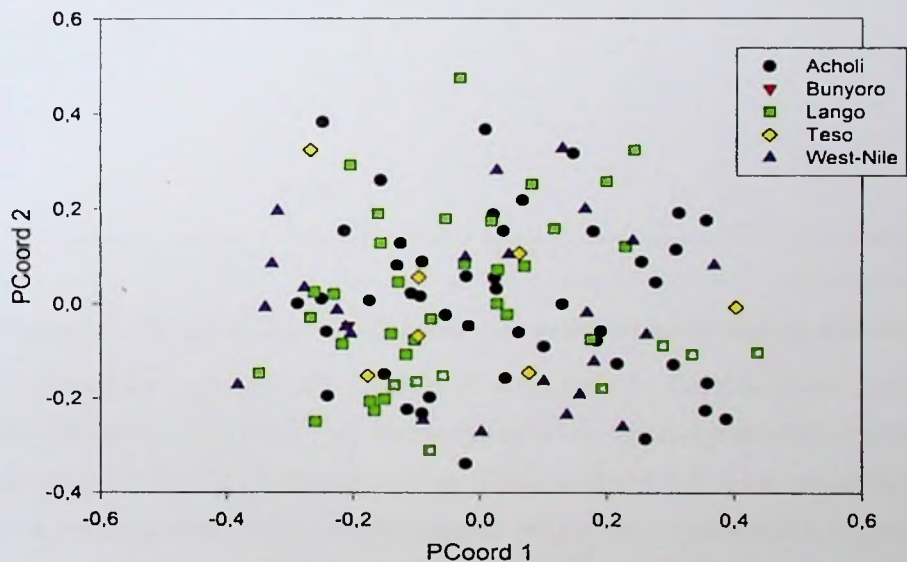


Fig 3.5: Genotypic diversity of landraces from different districts in Uganda.

The results showed that accessions from different sampled regions of Uganda seemed to be mixed without any pattern from one region to another indicating that seeds are transported from one region to another with no border and therefore the land races mix and do not show any clear difference except in phenotypic appearance of the land races. The land races therefore could not be grouped according to regions.

Principal coordinate analysis of the local land races grouped in districts revealed that the first two principal coordinates explained 38.35 % and 25.85 % of the variation, respectively (Figure 3.6). Abate *et al.* (2005) while studying diversity and population structure of 128 sesame land races in Ethiopia using 7 ISSR primers reported that clustering and principal coordinated analyses clustered the genotypes into groups where most of the land races were grouped in separate clusters irrespective of their geographic origins, while cultivars were grouped in one cultivar suggesting less variability within the released varieties than the land races.

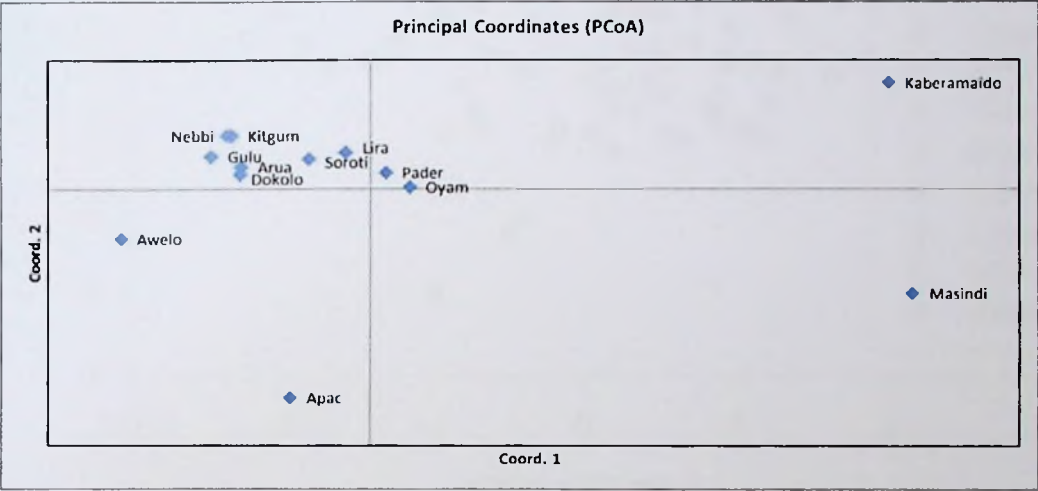


Figure 3.6: PCoA of the sesame landraces grouped according to districts.

A ‘core group’ including the districts of Arua, Dokolo, Gulu, Kitgum, Lira, Nebbi, Oyam, Pader and Soroti was identified. Accessions collected in Apac, Awelo, Kabermaido, and Masindi were grouped distantly from the core group. These separate 4 districts comprised a very little number of accessions each. Such a small number might have affected the statistical analysis and thus might have an impact on the resulting plot.

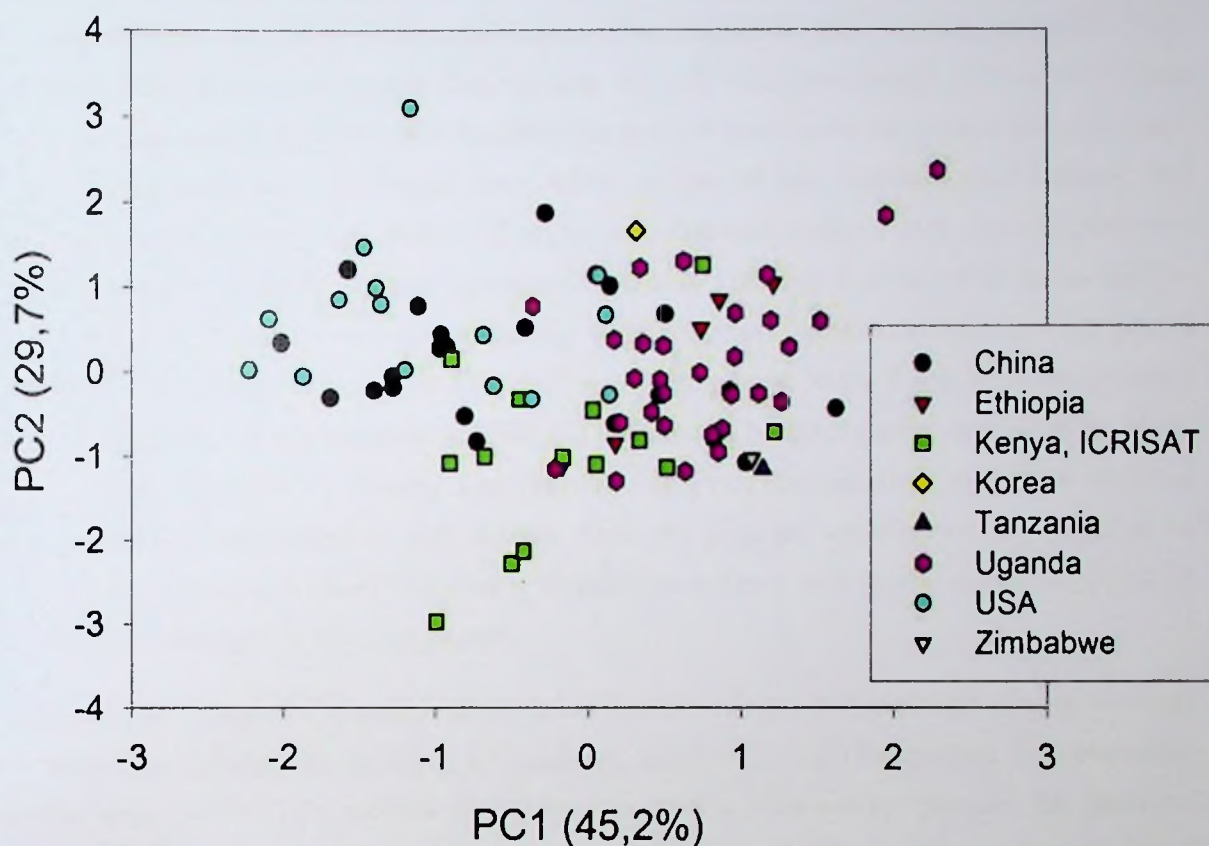


Figure 3.7: Principle component analysis based on 10 agromorphological traits of 84 Ugandan sesame lines

The Principal coordinate analysis of the agro-morphological traits of the 85 breeding lines is presented in Figure 3.7.

The accessions clustered in their respective country of origin and revealed a pattern associated to the main geographic origins. Clearly, the accessions from the USA overlap with those from China indicating their similarity. The accessions from Uganda overlapped with those from Ethiopia indicating similarity in their origin. The accessions from Kenya appear to be more distant although these Kenyan accessions from ICRISAT, did not indicate true origin of collection. The possibility of crossover events between accessions from different locations grown in the same field is high, knowing that cross pollination in sesame has been reported to occur at a frequency of between 5% and 60% (Wei *et al.* 2014).

3.4.4. Neighbour joining tree of the breeding lines

In order to resolve the relationships among the accessions, a NJ tree based on pairwise population matrix of Nei unbiased genetic distance values was generated (Figure 3.8). The lines, however, showed very little to no relationship with respect to their country of origin. Five accessions from Kenya maintained their genetic identity and relationship, but the remaining accessions were well intermixed. It is possible that the five accessions were from one origin and the intermixed must have originated from Africa or one of the countries near Uganda. No relationship according to their country of origin was detected; rather a vast commingling was visualized. Also the Sesim 2-related accessions 098, 114, 118, 191 from farmers' fields and the resulting cross 016, showed very high diversity. Kun Wu *et al.* (2014) examined the relatedness among 130 lines using 52 SSRs (39 EST-SSR and 13 genomoc SSRs) and 36 InDels using a model-based approach implemented in STRUCTURE and a NJ phylogentic tree based on Nei's genetic distance (Nei, 1972) whereby a neighbour-joining tree showed a tree with 5 branches but with 'mixed' lines distributed in each branch. Also, the principal coordinate analysis based on Nei's genetic distances showed five-cluster distribution pattern with mixed subgroups being in the middle of these five defined subgroups.

Furat *et al.* (2010) reported that regional isolation was not the sole factor contributing to diversity in a natural population. While using D^2 analysis, Saha *et al.* (2012) reported that clustering patterns indicated no strict patterns and there was narrow relationship between the observed genetic diversity and geographical differentiation, which showed that geographic diversity is not always related to genetic diversity.

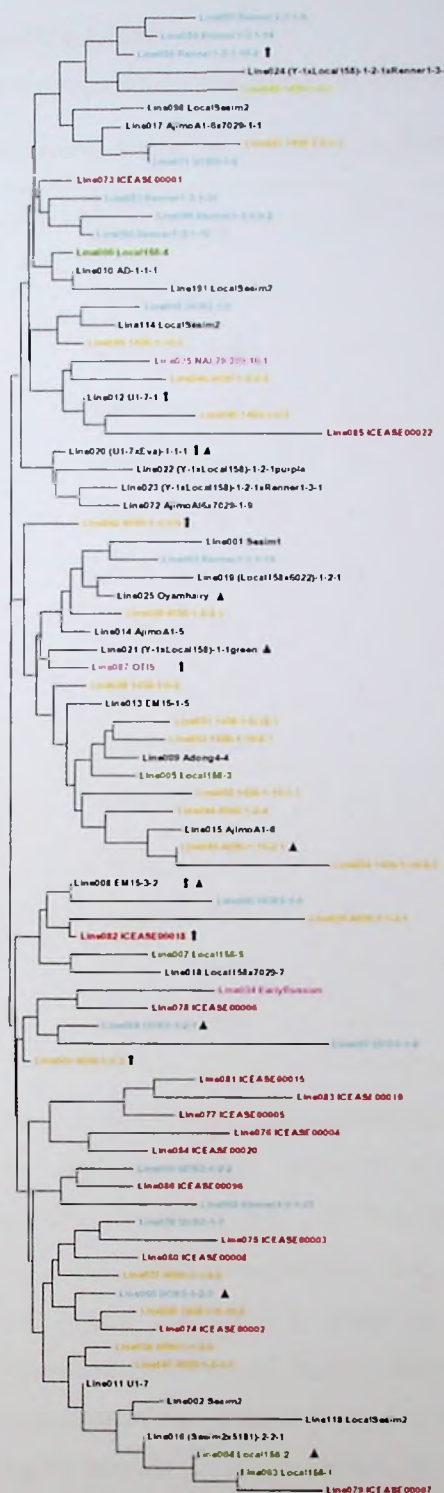


Figure 3.8: Neighbor-joining tree based on pairwise population matrix of Nei unbiased genetic distance for the sesame accessions

3.4.5. Private Alleles of the Breeding Lines

Summary of private (rare) alleles by population (breeding lines) is shown in Table 3.20.

Table 3.20: Summary of private (rare) alleles by population (Breeding lines)

	Population	Locus	Allele	Frequency
1	Line015_Ajimo A1-6	GBssr_sa_08	5	0.500
2	Line018_Local158x7029-7	CL569Contig1	3	0.281
3	Line044_4036-1-2-4	GBssr_sa_108	14	0.188
4	Line046_1438-1-6-1	GBssr_sa_123	5	0.031
5	Line053_1438-1-10-2-1	GBssr_sa_123	13	0.438
6	Line066_UCR-1-2-3	GBssr_sa_72	3	0.167
7	Line068_UCR-1-5	GBssr_sa_123	11	0.031
8	Line068_UCR3-1-5	GBssr_sa_184	3	0.031
9	Line070_UCR-1-7	GBssr_sa_184	11	0.031
10	Line076_ICEASE00004	GBssr_sa_184	4	0.500
11	Line080_ICEASE00008	GBssr_sa_184	12	0.500
12	Line084_ICEASE00020	GBssr_sa_108	15	0.125
13	Line085_ICEASE00022	CL569Contig1	4	0.844
14	Line085_ICEASE00022	GBssr_sa_08	11	0.031
15	Line085_ICEASE00022	GBssr_sa_108	16	0.031

Out of 85 breeding lines evaluated, only 13 lines were characterized by private alleles. These were four lines (ICEASE 00004, ICEASE 00008, ICEASE 00020, ICEASE 00022) which originated from ICRISAT, three lines (UCR3-1-2-3 , UCR3-1-5 and UCR3-1-7) from the University of California Riverside, USA; three lines (4036-1-2-4, 1438-1-6-1 and 1438-1-10-2-1) originated from China, one line (Local 158//7029-7) which is a cross between an accession from Ethiopia (Local 158) and Thailand (7029); and lastly a selected local line (Ajimo A1-6). Nuclear SSRs recorded a higher number of rare alleles compared to cytoplasmic SSRs. Komivi Dossa *et al.* (2016) while studying 96 sesame accessions (48 from nine African countries and 48 from 13 Asian countries) comprising land races and modern cultivars recorded that there were 137 alleles detected of which 49 (35.77%) were specific to geographical origin where West

Africa and East Asia exhibited the highest private allele (17 and 8, respectively) but no private alleles were found in West Asia which they felt, could have been due to low sample number from this area. They found that Africa harboured more private alleles (30) than Asia (21).

3.4.6. Outcrossing rates of sesame

From the seven different plants investigated, varying degrees of introgression by means of foreign pollination. The level ranged from 3.1 % (one seedling out of 32 of mother plant B), 12.5 % (one seedling out of eight of each of the mother plant A and C, D and E), 37 % (12 seedlings out of 32 of plant F), up to 59.4% (19 seedlings out out of 32 of plant G) of foreign alleles with a mean value of 28.1 %. In detail, 19 out of 32 investigated seeds had at least one or more foreign alleles as compared to the respective mother genotype per capsule and 9 investigated SSR loci. The detected natural cross-pollination explains the detected gene flow and demonstrates that controlled self-pollination is required to preserve genetic purity. Thus, efforts should be made to maintain sesame germplasm collections and breeding lines under controlled self-pollination or production of basic seed material under isolated conditions. Distribution of genetic diversity for a given species is dependent on evolutionary and breeding factors, species ecology, and geographical and human factors (Ramanatha and Hodgkin, 2002). In sesame, high local diversity is associated with cross-pollination, depending on the presence of pollinating insects at flowering time (Bhat *et al.*, 1999). However, large-scale diversity depends more strongly on human factors. Migration and trade have influenced sesame distribution hence; continuous gene flow can take place among cultivars from different geographical regions.

3.5 Conclusion

There was no pattern of distribution among the local landraces in Uganda but good distinction could be seen among the land-races, breeding lines and introductions. The markers separated the accessions among USA, China, breeding lines and local landraces. Most of the local landraces were also found mixed in different cultivars as shown by their mixed morphological characteristics probably due to trade in the different districts and regions. Farmers exchange seeds among themselves and also from the local markets thus resulting in varietal seed mixing.

CHAPTER FOUR

COMBINING ABILITY AND THE NATURE OF GENE ACTION CONTROLLING HYBRID YIELD PERFORMANCE IN SESAME

4.1 Abstract

The exploitation of genetically diverse parents in cross combinations is the principal breeding method to identify promising hybrids and also to develop superior pure lines from such crosses. Knowledge of the nature of the combining ability effects and their resulting variances has a paramount significance in deciding on the selection procedure for exploiting either heterosis or obtaining new desirable recombinants. The objectives of this study were to estimate general and specific combining abilities and assess the nature and magnitude of gene action controlling the inheritance of seed yield and some of its components in Uganda's sesame germplasm. Two different experiments were used. An 8 x 8 diallel cross mating design yielding 28F₁s was set up while the second experiment which was a line x tester cross mating design had 45F₁s and their 14 parents (nine local lines or females and five introduced genotypes used as testers or males). Experiment on diallel analysis was conducted only at the National Semi-Arid Resources Research Institute (NaSARRI), Serere, while the line x tester mating design experiment was carried out at both NaSARRI and NgeZARDI during the second season of 2013. The genetic variance of combining ability was separated into general (GCA) and specific (SCA) combining ability variance components. Baker's ratio, coefficients of genetic determination (CGD) were determined for narrow and broad sense heritabilities. Results showed that under the diallel mating design, high GCA effects were recorded for days to flowering, height of first capsule and number of branches, branches per plant and capsule length. Desirable SCA effects were recorded for Sesim2//5181x Renner 1-3-1-1 for reduced height of first branch (-9.48*), Sesim1 x Sesim2 for number of branches per plant (0.901*), capsules on branches (20.75**) and yield per plant (2.42*). High Baker's ratio and CGD_{bs} were recorded for most of the traits. These results suggested that both additive and non-additive gene actions played a great role on these traits. Sesim 1 had the highest number of desirable traits scored for GCA effects and could be considered as a parent for crossing to produce desirable progeny. In the line x tester mating design, the results showed that the mean squares across locations had high significant difference for all the traits indicating that locations had effect on the traits. Variances for the males were higher than the variances for the females for all the traits suggesting greater variability among

he males than the females. Combined general combining ability (GCA) across locations showed that GCAs for the lines and testers were significantly positive for a number of traits. Specific combining ability (SCA) was positively significant only for a few traits. Baker's ratio was high for most of the characters.. Coefficients of determination for narrow sense heritability (CD_{nsh}) were high for only three traits

Key words: Baker's ratio, coefficient of genetic determination, gene effects, hybrid vigor, heritability, dominance, additive effects

4.2 Introduction

Sesame is cultivated on 7.2 million ha. worldwide (FAO 2007) and mainly in developing countries of Africa and Asia. The productivity is low and so higher seed yields are a major breeding objective in sesame and highly depend on a number of traits.

The Uganda national yield of sesame is reported to be about 600 kg/ha (FAOSTAT, 2009) whereas Brigham (1985) reported average yields of 1,564 kg/ha in USA. This disparity in yields is caused by various constraints including lack of improved varieties. Although sesame is a self pollinated crop, interest has recently developed in producing hybrid varieties since a considerable amount of heterosis has been demonstrated suggesting that hybrid production appears to promise a yield breakthrough in sesame like in other self-pollinated crops such as rice, barley, wheat and could make sesame more competitive and lead to wider cultivation.

Successful breeding depends on the genetic variability among the available genotypes and the underlying gene action and genetic architecture of traits related to yield. It is very important to identify parents with high GCA values for the trait to be improved (Banerjee and Kole, 2009).

Information on gene action and combining ability helps in the choice of suitable parents for hybridization to develop superior F_1 hybrids so as to exploit hybrid vigour (Solanki and Gupta, 2001). Selection merely based on yield is not effective without knowledge on character association (Anitha *et al.*, 2010). Testing parents for their combining ability is very important because many times, the high yielding parents may not combine well to give good hybrids (Vavdiya *et al.*, 2014). The exploitation of genetically diverse parents in cross combinations is the principal breeding approach to identify promising hybrids and to develop superior pure lines from such crosses. Griffing (1956) provided an efficient estimation of combining ability and the

nature of gene action involved. General combining ability is largely due to additive genetic effects and additive x additive epistasis, while specific combining ability is largely a function of non-additive dominance and other types of epistasis. A more precise estimate of general combining ability and specific combining ability is by line x tester mating design (Kempthorne, 1957). Knowledge of the nature of the combining ability effects and their resulting variances has a paramount significance in deciding on the breeding selection procedure for identifying parents to exploit either heterosis or obtain new recombinants of desirable types in sesame (Solanki and Gupta., 2003).

In order to break the present yield barrier and evolve varieties with high yield potential, it is desirable to combine the genes from genetically diverse parents. The success in identifying such parents depends primarily on the nature of the gene action controlling the traits. Diallel and Line x Tester (L x T) analyses are used to select parents based on their combining ability.

The objective of the present study was to assess the nature and magnitude of gene action controlling seed yield and other yield traits in Uganda's sesame germplasm.

4.3. Materials and Methods

4.3.1. Experimental Sites

All the experiments were planted at the National Semi-Arid Resources Research Institute (NaSARRI), Serere district, eastern Uganda (Latitude 1°31'N; Longitude 33°27'E; and altitude 1,139 masl) during the second rains of 2013. The climate of Serere district is modified by the large swamp area surrounding it. The rain season is from March to November, with a marked minimum in June, and marked peaks in April to May for what is normally called the first rain season and August to October for the second rain season. The annual rainfall normally ranges from 1000 mm to 1500 mm. There is normally a short dry spell between the two rain seasons during mid-June-mid July. The long dry season sets in during late November through to early March. The mean annual maximum temperature is around 31.3°C and the minimum is 18°C. The soils are mainly of the ferrallitic type (sandy sediments and sandy loams which are friable and well drained. The second experiment on combining ability and gene effects using line x tester mating design had additional site at Ngetta Zonal Agricultural Research & Development Institute (NgeZARDI), Lira district in northern Uganda (latitude 2°17'N; longitude 32°56'E; altitude

.189 meters above sea level). The average annual rainfall in the district varies between 1200-600 mm decreasing northwards. The rainfall is mainly convectional and normally comes in the afternoons and evenings. The maximum temperature hardly goes beyond 36°C.

1.3.2 Study One: Diallel Mating Design

Eight diverse sesame genotypes including local and exotic lines (Table 4.1) that were previously characterized morphologically in the breeding programme at the National Semi-Arid Resources Research Institute and showed contrasting traits were crossed in a half 8 x 8 diallel mating design resulting in 28 F₁ combinations. These hybrids along with their parents were planted in a randomized complete block design in three replications at NaSARRI during second rains of 2013. The plots were single 2 m long rows with 30 x 10cm spacing. Sesim 2 variety was used as a border row planted at the beginning and the end of each replication to control the border effects. The genetic parameters were estimated according to Griffing's analysis of diallel crosses, Method 2 using model 1 (Griffing, 1956).

Table 4.1: Selected genotypes with their characteristics used in the diallel mating design

Parents	Country of origin	Days to flowering	Days to maturity	Height at first capsule (cm)	Branching habit	Plant height (cm)	Number of capsules per plant	Hairiness of capsules	Yield per plant (gms)
Sesim 1	Uganda	46-49	75-77	58-67	3-4	112-135	28-50	Absent	High
Sesim2	Uganda	48-49	76-78	46-73	3-4	107-1151	27-67	Absent	High
Ajimo A1-5	Uganda	51	76-77	55-64	3-5	107-116	34-58	Absent	High
Ajimo A1-6//7029	Cross between local and Thailand variety	44-48	76-80	45-70	2-3	99-120	35-44	Absent	High
Sesim2//5181	Cross between local and Thailand variety	Greater days	50	Middle	high	Tall	Many	present	High
SPS1438-1-6-4	China	35-36	76	22-29	0-2	101-1171	21-27	absent	Low
4036-1-10-2	China	46-49	76-77	52-53	2-3	104-1281	34-43	Present	Moderate
Renner 1-3-1-1	USA	45-48	76-79	55-69	2-3	115-128	22-40	Absent	Low

4.3.3 Study Two: line x tester mating design

4.3.3.1. Plant material

From the germplasm available which had been morphologically characterized at NaSARRI before this study, nine female local cultivars (Sesim 1, Sesim 2, Ajimo A1-5, Ajimo A1-6//7029, Adong 4-4, U1-7, EM15-1-5, Local 158//6022, Y-1//Local 158-1-2-1) were selected and used as female lines and crossed to 5 male tester accessions (Local 158, SPS1438-1-6-4, 4036-1-10-2, Renner 1-3-1-1, 7029-1-2) used as testers (Table 4.2) to produce 45 F₁s/hybrids. The 45 F₁s and the 14 parents (59 genotypes) were grown in a randomized complete block design (RCBD) with three replications. Each entry plot consisted of one 2m long row at spacing of 30 x 10cm.

Table 4.2: Characteristics of parents used as lines (females) and testers (males)

Parents	Country of origin	Days to flowering	Days to maturity	Height at first capsule	Height at first branch	Length of Capsule zone (cm)	No branches	Plant height (cm)	Hairiness of capsules
Females/Lines									
Sesim 1	Uganda	46-49	75-76	58-67	30-45	49-69	3-4	112-135	Absent
Sesim2	Uganda	48-49	76-78	46-73	26-48	42-59	3-4	107-115	Moderate
Ajimo A1-5	Uganda	51	76-77	55-64	26-42	42-54	3-5	107-116	Absent
Ajimo A1-6//7029	Cross between local and Thailand variety	44-48	76-80	45-70	29-46	54-65	2-3	99-123	Absent
Adong 4-4	Uganda	47-48	77-78	52-63	26-47	57-65	2-4	114-119	Absent
U1-7	Uganda	48-50	76-77	68-83	40-62	50-63	3-4	98-148	Absent
EM15-1-5	Uganda	48-49	76-78	64-71	37-38	56-74	4	118-148	Moderate
Local 158//6022	A cross between Ethiopian and Thailand line	44-48	76	47-68	36-40	38-52	2-4	97-122	Dense
(Y-1//Local 158)-1-2-1	A cross between unknown origin and Ethiopian	48-52	76-79	66-78	36-54	50-54	3-4	118-130	Absent
Males/testers									
Local 158	Ethiopia	46-49	76-80	62-68	40-44	42-56	2-3	103-119	Present
SPS1438-1-6-4	China	35-36	76	22-29	0	77-86	0-2	101-117	absent
4036-1-10-2	China	46-49	76-77	52-53	23-35	55-69	2-3	104-128	Present
Renner 1-3-1-1	USA	45-48	76-79	55-69	38-51	49-59	2-3	115-128	Absent
7029-1-2	Thailand	44-47	77	54-62	34-39	54-60	2-3	119-121	Absent

4.3.3.2. Data collection

The data recorded in all the studies were: number of days to 50% flowering, days to physiological maturity, number of primary branches per plant, height to first branch on the main stem, height to first capsule on the main stem, length of the capsule formation zone, number of capsules on the branches, number of capsules on the main stem, total number of capsules per plant, plant height, maturity and seed yield per plant on five randomly selected plants of each genotype in each replication.

4.3.4 Data analysis

4.3.4.1 Diallel mating design

The traits under study were subjected to analysis of variance for randomized complete block design on an entry mean basis. The combining ability analysis was carried out following Griffing's (1956) method 4 which includes only the direct F₁ crosses without parents and reciprocals. The estimates of GCA effect for the parents and the SCA effects for the crosses were calculated according to Singh and Chaudhary (1985).

The model followed was:

$$Y_{ij} = \mu + g_i + g_j + s_{ij} + \frac{1}{bc} \sum_k \sum_l \epsilon_{ijkl} \quad i,j=1,\dots,p.$$

Where:

Y_{ij} is the observation of cross (x_{ij}), μ is the population mean, g_i and g_j are the general combining ability effect for the i^{th} and j^{th} parents, S_{ij} is the specific combining ability effect of the cross between the i^{th} and j^{th} parents such that $S_{ij} = S_{ji}$ and ϵ_{ijkl} is the experimental error due to environmental effect associated with the $ijkl^{th}$.

The variance component was calculated using the formula: $\sigma^2_g = (MS_{gca} - MS_{error}) / (p-2)$;
 $\sigma^2_s = (MS_{sca} - MS_{error}) / 1$

where:

MS_{gca} = variance due to GCA; MS_{sca} = variance due to SCA; MS_{error} = Error variance

4.3.4.1.1 Gene action

Since the parents used in the crosses were considered fixed, coefficient of genetic determination were used to estimate total genetic variability, broad sense and narrow sense coefficient of genetic determination (CGD) by the formula below:

$$\text{Baker's ratio (Baker, 1978)} = (2 \sigma_{\text{gca}}^2) / (2 \sigma_{\text{gca}}^2 + \sigma_{\text{sca}}^2)$$

$$\text{CGD}_{\text{BS}} = (2 \sigma_{\text{gca}}^2 + \sigma_{\text{sca}}^2) / (2 \sigma_{\text{gca}}^2 + \sigma_{\text{sca}}^2 + \sigma_e^2)$$

$$\text{CGD}_{\text{NS}} = (2 \sigma_{\text{gca}}^2) / (2 \sigma_{\text{gca}}^2 + \sigma_{\text{sca}}^2 + \sigma_e^2)$$

4.3.4.2 Line x tester mating design

A line x tester mating design by Kempthorne (1957) and adopted by Singh and Chaudhary (1985) was followed, with its statistical model as:

$$Y_{ijkl} = \mu + a_i + b_{kl} + v_{ij} + (av)_{ijl} + \epsilon_{ijkl}, \text{ where:}$$

Y_{ijkl} is the observed value from each experimental unit (x_{ij}), μ is the population mean, a_i = location effect; b_{kl} = block or replication effect within each location; $v_{ij} = g_i + g_j + s_{ij}$, where g_i = general combining ability (GCA) for the i th parental line; g_j = GCA effect of j^{th} tester; s_{ij} = specific combining ability (SCA) for the ij^{th} hybrid and $(av)_{ijl}$ = interaction effect between ij^{th} F_1 hybrid and l^{th} location; and ϵ_{ijkl} = residual effect.

The means of the characters for the different entries were subjected to L x T analysis and variance due to general combining ability (GCA) of parents and specific combining ability (SCA) of different cross combinations were worked out on the procedure developed by Kempthorne (1957) and demonstrated by Singh and Chaudhary (1985).

The significance of mean square for line x testers provides a direct test of significance of dominance variance, σ_D^2 while significance of σ_A^2 is provided by significance of lines and testers mean squares.

4.4 Results and Discussion

4.4.1 Study One: Combining Ability and Gene Action in Sesame Elite Genotypes by Diallel Mating Design

4.4.1.1. General combining ability effects

The resulting general combining ability (GCA) values of the parents are presented in Table 4.3. High positive significant ($P < 0.001$) GCA effects were recorded for days to flowering in Sesim 1, Sesim 2, Ajimo A1-5 and Sesim2//5181. These genotypes could enhance yield through these traits that contribute positively to yield. Highly negative significant ($P < 0.001$) GCA effects were recorded for the same traits in Ajimo A1-6//7029 and SPS1438-1-6-4. With change in rainfall pattern getting reduced and being unpredictable, reduced time of flowering will also reduced time of maturity as they are positively correlated.

Table 4.3: General combining ability effects for various yield components in Sesame

Parents	Days to Flower	Plant height (cm)	Height of first branch (cm)	Height 1st capsules (cm)	Branches/ plant	Capsules on main stem	Capsules on branches	Length of capsule zone (cm)	Yield/ Plant (gm)
Sesim 1	1.44***	5.09*	-6.49***	-3.65*	0.81***	0.69ns	7.57*	6.26*	0.39ns
Sesim 2	2.33***	3.59ns	5.02*	9.45***	0.42*	-1.36ns	3.77ns	-3.88ns	0.21ns
Ajimo A1-5	1.66***	5.26*	0.57ns	4.85**	0.58**	0.12ns	7.46*	1.36ns	0.30ns
Sesim2/5181	1.72***	-0.25ns	8.52***	8.61***	-0.34ns	-3.21**	-6.51*	-10.71***	0.26ns
Ajimo A1-6/7029	-1.52***	-3.44ns	0.99ns	-0.56ns	-0.40*	-3.07*	-0.04ns	-2.94ns	0.47ns
SPS1438-1-6-4	-4.48***	-3.53ns	-7.28***	-11.83***	-0.86***	-2.69*	-6.34*	9.01**	0.45ns
4036-1-10-2	0.16ns	-1.31ns	0.86ns	-1.88ns	-0.25ns	1.53ns	-4.96ns	0.73ns	0.29ns
Renner 1-3-1-1	-1.29**	-5.80*ns	-2.96ns	-5.61**	0.11ns	1.68ns	0.35ns	-0.47ns	-0.29ns
SE _{GCA}	0.37	2.49	1.97	1.78	0.19	1.20	2.91	2.68	0.49

Significant positive ($P < 0.05$) GCA effects were recorded for plant height in Sesim 1 and Ajimo A1-5 only. Plant height is positively correlated to yield in sesame and so these genotypes could play a role in improving yield in the breeding programme.

Highly significant negative effect ($P < 0.001$) was recorded for height of first branch in Sesim 1 and SPS1438-1-6-4. Low height of first branch meant that more capsules will start setting nearer to the ground, as more branches per plant are formed. Branching habit has been found to be positively correlated to yield per plant (Ahadu, 2008).

Highly positive significant ($P < 0.001$) GCA effects was recorded for number of branches per plant in Sesim 1 and high positive significant ($P < 0.01$) effect in Ajimo A1-5. A higher number of branches is positively correlated to yield per plant.

Significant positive ($P < 0.05$) GCA effect were recorded for number of capsules on branches in Sesim 1 and Ajimo A1-5. A higher number of branches leads to higher seed yield/plant.

High positive significant ($P < 0.01$) GCA effect and significant ($P < 0.05$) GCA effect for length of the capsule zone were recorded for genotypes SPS1438-1-6-4 and Sesim 1 respectively. The longer the length of the capsule zone, the greater the number of capsules set if the internode length of capsules are narrow (Teklu *et al.* 2017).

No genotype contributed to higher GCA effect for yield per plant but Ajimo A1-6//7029, SPS1438-1-6-4, Sesim 1 and Ajimo A1-5 though not significant, tended towards contributing positively to better yield/plant. Vavdiya *et al.* (2014) reported that none of the parents was a good general combiner for all the characters studied and this is similar to the findings of this study..

Sesim 1 was the best parent with the highest number of significant GCA effects followed by SPS1438-1-6-4. Thirugnana *et al* (2006) reported significant results for general combining ability for all the characters in sesame when evaluated under both normal and flood environments except for 1000 seed weight under normal conditions. Significant results of GCA suggest important role of additive genetic effects in the inheritance of these characters.

Ravindran and Raghinam (1996) reported predominance of additive gene effects in traits like branches per plant, days to 50% flowering, 1000 seed weight and height to first capsule. Early flowering could be a desirable selection criterion if the reproductive period was long enough to increase productivity or if the shorter time to flowering resulted in a concomitant decrease in time to maturity without decreasing the yield significantly or if it helps escape the terminal drought.

4.4.1.2. Specific combining ability effects

The estimates of specific combining ability (SCA) are presented in Table 4.4. Ajimo A1-5 x Renner 1-3-1-1 had positive significant ($P<0.05$) effect for the height of first branch (10.83) which is not desirable because few capsules would then be set on the main stem thus resulting in less yield per plant and more so if few or no branches are developed. Meanwhile Sesim 2//5181 x Renner 1-3-1-1 had negative significant ($P<0.05$) SCA effect (-9.48) which is desirable as the capsules start developing from the lower part of the main stem and therefore more capsules are formed on the plant. Sesim 1 x Sesim 2, Sesim 1 x Ajimo A1-5 and Sesim 2//5181 x SPS 1438-1-6-4 showed positive significant ($P<0.05$) effect for yield per plant. Sesim 1 x Sesim 2 had positive significant ($P<0.05$) SCA effect for days to flowering (1.59), number of branches per plant (0.90), number of capsules on branches (20.75). This cross could be a good hybrid as those traits contribute positively to yield per plant. None of the hybrids exhibited superior SCA effects for all the characters indicating that none of the crosses was superior for all the traits.

Vavdiya *et al.* (2014) reported that the good general combiners when crossed may not always produce the best hybrid. They argued that this could be attributed due to lack of complementation between favourable alleles of the parents involved.

Table 4.4: Estimates of specific combining ability effects for yield and yield components

Cross	Days to flowering	Maturity	Height 1 st branch (cm)	Height 1 st capsules (cm)	Plant height (cm)	Branches/ plant	Capsules on main stem	Capsules on branches	Capsules zone (cm)	Yield/ plant (gm)
4036-1-10-2 x Renner 1-3-1-1	-0.19	0.71	1.54	5.94	5.33	-0.06	-0.08	0.02	-1.58	0.41
Ajimo A1-5 x 4 036-1-10-2	-0.13	-0.03	-2.65	0.48	-0.02	0.134	-0.85	3.57	1.25	-0.5
Ajimo A1-5 x Ajimo A1-6//7029	-0.12	0.21	-3.79	-3.52	1.11	-0.38	1.41	7.99	4.26	-1.28
Ajimo A1-5 x Renner 1-3-1-1	-0.02	0.01	10.83*	7.2	2.46	-0.22	-1.34	-2.06	-3.88	0.8
Ajimo A1-5 x Sesim2//5181	-0.02	-0.63	4.33	1.32	-6.09	-0.78	-4.45	-14.88*	-5.3	-2.62*
Ajimo A1-5 x SPS1438-1-6-4	1.18	1.2	-3.66	1.08	7.89	0.41	2.31	9.63	6.64	2.01
Ajimo A1-6//7029 x 4036-1-10-2	0.38	0.24	-2.08	-6.46	-3.63	0.11	0.67	-0.59	3.89	0.76
Ajimo A1-6//7029 x Renner 1-3-1-1	2.17**	-0.38	-1.26	-0.06	-0.14	0.09	0.19	-4.56	1.09	0.23
Ajimo A1-6//7029 x SPS1438-1-6-4	-0.98	-0.20	5.71	3.83	-6.41	0.39	-5.5*	-5.2	-11.06	-1.43
Sesim 2 x 4036-1-10-2	0.37	-0.77	3.22	3.2	-4.66	-0.04	-3.04	-8.06	-7.51	-0.66
Sesim 2 x Ajimo A1-5	0.37	-0.80	2.51	-2.2	-4.92	0.13	-1.63	-10.15	-6.13	-0.70
Sesim 2 x Ajimo A1-6//7029	-1.46	0.14	1.41	6.21	9.07	-0.22	3.23	2.36	1.83	1.73
Sesim 2 x Renner 1-3-1-1	-0.69	0.28	-4.63	-6.4	-3.17	-0.06	0.48	3.96	2.36	-1.39
Sesim 2 x Sesim2//5181	0.30	0.60	8.21	6.71	5.58	0.05	1.03	2.82	-2.39	-0.67
Sesim 2 x SPS1438-1-6-4	-0.50	0.13	-2.97	-5.18	-8.44	-0.76	-1.2	-11.67	1.21	-0.71
Sesim 1 x 4036-1-10-2	0.74	0.57	7.07	2.64	-4.56	-0.43	-2.08	-8.86	-5.65	-2.02
Sesim 1 x Ajimo A1-5	-1.26	0.05	-7.57	-4.36	-0.42	0.70	4.55	5.9	3.16	2.28*
Sesim 1 x Sesim2	1.59*	0.41	-7.76	-2.35	6.54	0.90*	1.13	20.75**	10.63	2.42*
Sesim 1 x Sesim2//5181	-0.13	-0.43	5.39	0.15	-4.22	-0.34	-4.68	-6.31	-10.54	-1.26
Sesim 1 x SPS1438-1-6-4	-0.94	-0.61	2.87	3.92	2.66	-0.82*	1.08	-11.47	2.41	-1.40
Sesim2//5181 x 4036-1-10-2	-0.86	0.39	-5.29	-2.28	6.48	0.05	3.14	7.54	10.66	1.53
Sesim2//5181 x Renner 1-3-1-1	-1.07	-0.56	-9.48*	-6.22	-4.73	0.36	2.33	0.57	3.86	0.97
Sesim2//5181 x SPS1438-1-6-4	1.78*	0.63	-3.16	0.32	2.99	0.66	2.64	10.26	3.71	2.07*
SPS1438-1-6-4 x 4036-1-10-2	-0.33	-1.10	-1.81	-3.52	1.06	0.24	2.24	6.38	-1.06	0.49
SPS1438-1-6-4 x Renner 1-3-1-1	-0.216	-0.06	3.01	-0.46	0.25	-0.12	-1.57	2.07	-1.86	-1.02
SE _{SCA}	0.78	0.61	4.13	3.72	5.21	0.40	2.51	6.09	5.60	1.21

*, ** Significant at 5% and 1% probably level respectively

4.4.1.3. Combining Ability Variances, Baker's Ratio and Coefficient of Genetic Determination for Diallel Mating Design.

GCA and SCA variances, Baker's ratio and coefficient of genetic determination are presented in Table 4.5. GCA variances were found to be highly significant ($P<0.001$) for days to flowering, branches per plant, length of capsule zone and significant ($P<0.05$) for plant height and capsules on main stem indicating preponderance of additive gene action. SCA showed significant effect for capsules on branches and yield per plant thus indicating major action of non-additive gene action for these traits. Saravanan and Nadarajan (2003) reported that the combining ability for 13 traits revealed that the general combining ability and specific combining ability were significant for all the characters studied but the GCA variance was greater than the SCA variance for eight traits including days to 50% flowering, plant height and number of primary branches per plant indicating preponderance of additive gene action for the traits while the SCA variance was greater than GCA variance for number of capsules per plant, single plant yield indicating predominance of dominant gene action for these traits. Murty (1975) reported that general combining ability variance was larger than specific combining ability variance for all the characters except oil content, indicating the predominance of additive gene action. Thirugnana *et al* (2006) reported that the magnitude of GCA variance was higher than that of SCA variance for days to 50 per cent flowering and plant height under normal conditions. This study showed that additive gene action controlled days to flower, plant height, branches per plant, capsules on main stem, capsules on branches and length of capsule zone. Non-additive gene action controlled capsules on branches and yield per plant only. The selection of desirable traits that are controlled by additive gene action could be done at early stages of segregation to produce pure lines. Meanwhile, traits controlled by non-additive (dominance and epistasis) gene action are good for hybrid production where dominance is important.

Baker's ratio was high for most of the traits (Table 4.5) indicating that the parents contributed much to the progenies. CGDBs and CGDNs were high for most traits such as days to flowering, height to first branch, branches per plant and length of capsule zone implying that these traits have high heritability and the parents contributed highly to their offsprings. Mothilal and Manoharan (2005) reported high heritability for plant height and number of branches per plant, moderate heritability for number of capsules on branches while low heritability were reported for number of capsules on main stem, and seed yield per plant.

Table 4.5: General (GCA) and specific (SCA) combining ability variances, Baker's ratio and coefficient of genetic determination in broad and narrow sense.

	Days to flower	Maturity	Height 1st branch (cm)	Plant height (cm)	Branches/ plant	Capsules on main stem	Capsules on branches	Capsule zone (cm)	Yield/ plant (gm)
GCA	31.93***	0.65ns	144.91ns	105.7*	1.71***	22.42*	178.01**	191.17***	3.01ns
SCA	1.25ns	0.44ns	39.58ns	34.89ns	0.28ns	9.68ns	99.96*	45.71ns	2.97*
Baker's ratio	0.96	1.0	0.70	1.0	0.89	0.82	0.44	0.96	0.23
CGD _{bs}	0.92	0.06	0.69	0.356	0.69	0.36	0.62	0.51	0.57
CGD _{ns}	0.88	0.06	0.48	0.356	0.61	0.30	0.27	0.49	0.13

4.4.2. Study Two: Combining ability and gene action based on line x tester mating design

4.4.2.1. General combining ability gene effects for Serere

General combining ability effects for various yield components in sesame at Serere are presented in Table 4.6. Within the lines, Em15-1-5 ($P<0.01$), Ajimo A1-5 ($P<0.05$), and Sesim 2 ($P<0.05$) had significant positive GCA effects for days to flowering. These parents therefore could be used for increasing the number of days for flowering. Meanwhile, Local158//6022 and Ajimo A1-6//7029 had highly significant ($P<0.001$) negative GCA effects and therefore they could be used to reduce time of flowering especially with the present change in climate in order to reduce drought effect at flowering. Babu *et al.* (2004) reported the importance of additive type of gene action for days to 50% flowering.

Positive significant GCA effect ($P<0.05$) was recorded for Ajimo A1-5 for days to maturity and negative significant effect for Sesim 1 and Y-1//Local158-1-2-1. So, Ajimo A1-5 could be used for increasing number of days to maturity in some genotypes that may again be so early maturing such that the yields are compromised.

For height to first branch, highly positive significant effect ($P<0.001$) was recorded for Y-1//Local158-1-2-1 and high negative significant effect ($P<0.01$) for Local 158//6022. Y-1//Local158-1-2-1 would not be a desirable parent because increasing the height of first branch would mean increasing the height of the first capsule on the main stem as well since first capsule normally sets above the first branch on the main stem. Local 158//6022 would be a desirable parent as capsules would also be set lower on the stem and possibly with more capsules.

High positive significant effect ($P<0.01$) for height to first capsule was recorded in Ajimo A1-5 and Y-1//Local 158-1-2-1 and high negative significant effect ($P<0.01$) in local 158//6022. Since there is positive correlation between height of first branch and height of first capsule, branching high up on the stem would also start setting capsule high up on the stem. These are not desirable traits because fewer capsules would be formed on the main stem.

Table 4.6: General combining ability effects for various yield components in sesame at Serere

Genotypes	Days to flowering	Days to maturity	Height of 1 st branch (cm)	Height of 1 st capsule (cm)	Plant height (cm)	Number of branches per plant	Number capsules branches	Number capsule mainstem	Total number capsules	Length of capsule zone (cm)	Yield per plant (gms)
Lines											
Sesim 1	-0.178	-0.633*	-1.521	-0.548	3.683	0.122	-1.317	0.909	-0.779	3.887	0.408
Sesim 2	0.821*	0.112	-0.121	-0.208	-0.797	0.055	-0.757	-0.621	-2.239	-0.643	-0.502
Ajimo A1-5	0.887*	0.632*	0.928	5.191**	1.163	0.095	1.342	-1.291	-1.379	-3.513	-0.690
Ajimo A1-6/7029	-1.646***	-0.167	-0.121	-2.078	-2.257	-0.411	-1.977	-0.891	-2.109	-0.653	0.479
EM15-1-5	1.219**	0.290	2.068	-0.268	2.823	-0.302	-0.807	1.839	1.240	2.957	0.375
U1-7	0.627	0.546	-1.121	-1.078	0.483	-0.144	2.082	2.309**	3.960	2.217	0.987**
Adong 4-4	-0.183	0.390	-1.121	-1.878	-2.937	-0.279	-4.377*	-1.221	-6.309*	-0.113	-1.487***
Local 158/6022	-1.959***	-0.427	-6.421**	-4.818*	-3.857	0.348	7.162**	4.589***	14.160***	0.967	1.651***
(Y-1/2)Local 158-1-2-1	0.021	-0.831**	6.148***	4.721*	0.463	0.588**	0.082	-2.351*	-3.709	-4.913	-0.984**
S.E	0.388	0.287	1.841	1.850	2.545	0.216	2.063	1.120	2.829	2.652	0.366
Testers											
Local 158	1.371***	0.433	6.66***	8.208***	3.567	0.314	2.830	-0.781	2.687	-4.520*	-0.495
SPS1438-1-6-4	-2.972***	-0.570**	-4.88***	-8.081***	-4.133*	-0.531***	-4.789**	-0.271	-7.242***	4.109*	-0.608*
4036-1-10-2	1.103***	0.492*	-1.14	-0.971	2.937	-0.048	0.400	1.835**	3.437	3.959*	0.247
Remer 1-3-1-1	0.770**	-0.078	-0.17	-0.602	-3.873*	0.224	0.710	-0.341	0.467	-3.820	0.274
7029-1-2	-0.120	-0.228	0.27	2.358	1.897	0.077	1.160	-0.531	0.947	-0.230	0.537
S.E	0.289	0.214	1.372	1.379	1.896	0.161	1.538	0.835	2.109	1.976	0.292

*, **, *** Significant at 5%, 1% and 0.1% Probability level.

formed on those branches. The number of branches is also correlated to seed yield indirectly by affecting the number of capsules per plant. Mothilal and Manoharan (2005) reported that number of branches was governed by additive gene action.

High positive significant ($P < 0.01$) effect was recorded in local 158//6022 for number of capsules on branches. Positive significant effect for the number of capsules on branches indicates better yields as it is positively correlated to seed yield per plant.

Highly positive significant effect and positive effect ($P < 0.05$) were recorded in Local 158//6022 and U1-7 respectively for number of capsules on main stem. Number of capsules on the main stem is also positively correlated with seed yield per plant. Therefore, Local 158//6022 and U1-7 would be good selections as female parents for crossing.

For total number of capsules per plant, highly significant positive effect ($P < 0.001$) was recorded in Local 158//6022. Due to positive correlation between yield per plant and the number of total capsules per plant, Local 158//6022 is therefore a better parent (Ahadu, 2008).

For yield per plant, Local 158/6022 had highly positive significant effect followed by U1-7. Local 158/6022 and U1-7 are desirable to improve yield per plant since they show direct yield potential. Das and Choudhary (1998) reported that there was predominance of non-additive gene effects for yield per plant.

From the results presented, Local 158/6022 recorded seven significant traits of importance for improvement among the lines and, therefore, could be selected as the best female line for improving traits of other genotypes. This was followed by Ajimo A1-6//7029 and U1-7 with four and three desirable traits respectively.

Under testers, highly positive significant effect ($P < 0.01$) was recorded in Local 158 and 4036-1-10-2 and high positive significant effect ($P < 0.01$) in Renner 1-3-1-1 for days to flowering. Highly negative significant difference ($P < 0.01$) was recorded in SPS1438-1-6-4. High significant negative effect in SPS1438-1-6-4 and significant positive effect ($P < 0.05$) in 4036-1-10-2 were recorded for days to maturity.

Highly positive significant effect in Local 158 and highly negative effect was observed in SPS1438-1-6-4 for height of first branch and height of first capsule. Local 158 is therefore not desirable for height of first branch as it would reduce the number of capsules on the main stem while SPS1438-1-6-4 would be desirable to reduce height of first branch and first capsule. By reducing the height of first branch and first capsule, more capsules are formed on the main stem so long as the plant height is maintained or increased. This would also increase seed yield per plant as there would be more capsules on the main stem that contributes directly to yield.

SPS1438-1-6-4 and 4036-1-10-2 had positive significant effect for length of capsule zone. Therefore, SPS1438-1-6-4 and 4036-1-10-2 could be desirable parents for increasing seed yield through increase in the number of capsules on the main stem as the length of the capsule zone is increased.

Yield per plant had SPS1438-1-6-4 recording negative significant effect. Low number of branches in SPS1438-1-6-4 contributed to its low seed yield per plant.

4.4.2..2 Estimates of specific combining ability effects for Serere

Estimates of specific combining ability effects for yield and yield components in sesame at Serere are presented in Table 4.7. For height of first branch, significant positive SCA effect ($P<0.05$) was recorded in EM15-1-5 x Local 158 suggesting dominance gene action.

For plant height, significant SCA negative effects ($P<0.05$) were recorded for Sesim 1 x SPS1438-1-6-4. Number of branches per plant had only Local 158//6022 x SPS1438-1-6-4 with high positive significant effect ($P<0.05$).

Meanwhile for length of capsule zone, Sesim 1 x SPS1438-1-6-4 and EM15-1-5 x Renner 1-3-1-1 had significant ($P<0.05$) and ($P<0.01$) positive SCA effect respectively. No cross recorded any significant effect for seed yield per plant although dominance plays a great role in seed yield. Mothilal and Manoharan (2005) reported non-additive gene action was involved in the expression of number of capsules on branches and seed yield.

Cross	Days flower	Days maturity	Height 1 st branch (cm)	Height 1 st capsule (cm)	Plant height (cm)	Number branches per plant	Number capsules on branches	Number capsules on mainstem	Total number of capsules	Length of capsule zone (cm)	Yield per plant (gm)
1 Sesim 1 x Local 158	0.80	-0.49	1.95	1.11	-5.23	0.43	-6.23	-3.03	-8.10	-5.32	-1.00
2 Sesim 1 x SPS1438-1-6-4	-1.20	1.18	-3.83	-0.94	13.17*	0.27	10.39*	5.79*	11.83	12.06*	1.96*
3 Sesim 1 x 4036-1-10-2	-0.28	-0.88	0.42	-3.05	-4.90	-0.21	-4.14	-2.32	-6.51	-4.13	-1.28
4 Sesim 1 x Renner 1-3-1-1	0.40	0.69	0.12	3.58	4.51	-0.15	3.22	3.19	7.80	2.32	0.86
5 Sesim 1 x 7029-1-2	0.29	-0.49	1.34	-0.71	-7.55	-0.34	-3.23	-3.62	-5.02	-4.94	-0.58
6 Sesim 2 x Local 158	0.80	0.43	-2.78	-5.56	-0.45	-0.17	-0.46	1.50	1.37	5.22	0.79
7 Sesim 2 x SPS1438-1-6-4	-0.86	-0.24	0.77	1.40	6.25	-0.33	-0.70	1.65	1.97	5.93	-0.46
8 Sesim 2 x 4036-1-10-2	-0.28	-0.30	1.35	3.62	-4.42	0.19	7.21	-4.79	-10.38	-9.26	-0.79
9 Sesim 2 x Renner 1-3-1-1	1.40	-0.06	4.05	1.92	-2.31	-0.08	-2.68	-1.61	-3.07	-2.15	0.46
10 Sesim 2 x 7029-1-2	-1.05	0.16	-3.39	-1.38	0.93	0.40	7.21	3.25	10.11	0.26	0.20
11 Ajimo A1-5 x Local 158	0.06	0.91	-1.83	0.38	8.29	0.45	8.44	3.83	8.83	7.75	1.78
12 Ajimo A1-5 x SPS1438-1-6-4	-0.60	-0.76	1.97	-0.33	-8.31	-0.50	-5.30	-2.01	-7.24	-0.54	-0.96
13 Ajimo A1-5 x 4036-1-10-2	-0.34	-0.15	1.64	0.55	1.62	-0.18	-2.14	0.55	0.43	2.60	0.70
14 Ajimo A1-5 x Renner 1-3-1-1	0.33	0.42	-4.67	1.85	0.43	0.54	4.56	-2.61	0.73	-1.28	-0.62
15 Ajimo A1-5 x 7029-1-2	0.55	-0.42	2.89	-2.45	-2.03	-0.31	-5.55	0.25	-2.75	-0.54	-0.90
16 Ajimo A1-6/7029 x Local 158	-3.08**	-0.62	-3.78	-5.36	-3.59	-0.37	-3.24	0.10	-3.77	0.22	0.10
17 Ajimo A1-6/7029 x SPS1438-1-6-4	0.61	-0.29	-0.90	-2.73	0.11	-0.20	-0.28	1.59	1.84	2.59	-0.06
18 Ajimo A1-6/7029 x 4036-1-10-2	1.19	-0.02	3.02	2.15	-4.36	0.32	-1.47	-2.19	-4.18	-0.26	-0.76
19 Ajimo A1-6/7029 x Renner 1-3-1-1	0.19	0.22	-0.62	1.79	8.15	0.38	9.22*	1.66	11.46	5.46	1.32

		branch (cm)	capsule (cm)	per plant (cm)	on branches	on mainstem	of capsules	zone (cm)
20	Ajimo A1-6/7029 x 7029-1-2	2.28	4.15	-0.31	-4.23	-1.15	-5.35	-0.41
21	Adong 4-4 x Local 158	0.89	9.78	4.69	4.83	-1.90	3.10	-2.32
22	Adong 4-4 x SPS1438-1-6-4	-3.57	-2.26	3.39	-2.88	3.25	2.36	6.73
23	Adong 4-4 x 4036-1-10-2	3.35	3.95	0.02	-2.41	-1.85	-5.64	-2.13
24	Adong 4-4 x Renner 1-3-1-1	3.72	-6.09	-7.47	-3.05	-1.67	-5.67	-7.02
25	Adong 4-4 x 7029-1-2	-4.39	-5.38	-0.63	3.50	2.18	5.85	4.73
26	UI-7 x Local 158	-2.11	-2.36	-5.03	3.04	-2.10	0.84	-1.98
27	UI-7 x SPS1438-1-6-4	-0.90	-4.74	5.37	-5.68	3.06	0.43	9.73
28	UI-7 x 4036-1-10-2	1.69	3.15	2.90	-0.88	0.61	0.42	0.87
29	UI-7 x Renner 1-3-1-1	-1.49*	3.45	-8.59	-8.19	-4.54	-13.93*	-12.68*
30	UI-7 x 7029-1-2	0.66	0.49	5.35	11.71**	2.98	12.24	4.06
31	EM15-1-5 x Local 158	8.03*	4.50	-2.67	-2.41	-1.96	-3.45	-8.39
32	EM15-1-5 x SPS1438-1-6-4	-0.42	-2.54	-10.97	-1.79	-4.81	-8.52	-9.01
33	EM15-1-5 x 4036-1-10-2	-3.90	-2.95	0.96	6.88	3.06	11.23	1.49
34	EM15-1-5 x Renner 1-3-1-1	-11.47**	-6.02	5.07	3.38	5.26*	9.44	14.25**
35	EM15-1-5 x 7029-1-2	7.75	7.02	7.60	-6.07	-1.55	-8.70	1.65
36	Local 158//6022 x SPS1438-1-6-4	0.30	7.34	-9.99	-2.42	-5.22*	-6.10	-16.68**
37	Local 158//6022 x 4036-1-10-2	-0.42	-6.77	1.24	6.05	4.00	7.88	8.13
38	Local 158//6022 x Renner 1-3-1-1	0.15	-0.80	4.46	-1.92	1.52	1.19	4.57
39	Local 158//6022 x 7029-1-2	-0.60	0.24	4.29	-1.71	-0.30	-2.96	3.98
40	(Y-1//Local 158)-1-2-1 x Local 158	-0.63	-2.49	3.99	-3.96	3.56	1.17	4.82
42	(Y-1//Local 158)-1-2-1 x 4036-1-10-2	0.32	-4.58	6.92	1.47	2.94	6.76	10.67
43	(Y-1//Local 158)-1-2-1 x Renner 1-3-1-1	-0.44	6.79	-4.27	-4.54	-1.20	-7.94	-3.88
44	(Y-1//Local 158)-1-2-1 x 7029-1-2	0.71	-3.33	-7.63	-1.63	-2.02	-3.42	-8.81
SE		0.87	4.12	5.69	4.62	2.50	6.33	5.93

*, **, *** Significant at 5%, 1% and 0.1% Probability level.

4.4.2.3. General and specific combining ability variances at Serere

Results of the general (GCA) and specific (SCA) combining ability variances for yield and some of its components at Serere are presented in Table 4.8. Highly positive significant ($P < 0.01$) GCA variances for lines were observed for days to flowering, height of first branch, number of capsules on main-stem and total number of capsules. GCA variances for testers were highly significant ($P < 0.01$) for days to flowering and height of first capsule, high significance ($P < 0.5$) for days to maturity, height of first branch and total number of capsules implying that these testers contributed highly for the variation in these traits. GCA variances for testers were higher than GCA variances for lines for all the traits except number of capsules on main stem, 1000 seed weight, number of seeds per capsule and yield per plant suggesting higher GCA variances for these testers and therefore contributing to the variability in these traits. Hassaballa *et al.* (1996) and Shabana *et al.* (1996) reported that both general and specific combining ability variances were highly significant for all the traits they studied except oil percentage.

The SCA showed highly significant SCA variances ($P < 0.001$) for capsule length while significant effect ($P < 0.01$) was observed for number of capsules on main-stem, yield per plant and significant effect ($P < 0.5$) for days to flowering, number of capsules on branches, total number of capsules, length of capsule zone and number of seeds per capsule suggesting predominance of non-additive effects for these traits. These traits could be useful for hybrid seed development as suggested by Mishra & Sikarwar (2001). GCA was more important than SCA in almost all the traits which clearly were governed by additive gene action. Kumar and Ganesh (2002) while analyzing five lines crossed to three testers reported that dominant gene action was predominant for most of these traits. Hamouda (2001), Hoballah *et al.* (2001) and Krishna *et al.* (2002) concluded that general and specific combining ability mean squares were found to be highly significant for all the traits they studied.

Baker's ratio (BR) showed that high ratios were recorded for a number of traits (Table 4.8) suggesting that parents contributed highly in the crosses to these traits (Baker, 1978). The coefficients of determination due to broad sense heritability were also high for these traits indicating that the parents contributed highly to the traits (Bernardo, 2010).

Table 4.8: General (GCA) and specific (SCA) combining ability variances, Baker's ratio and coefficient of genetic determination for yield and its components at Serere.

Parameter	D.F	Days to flower	Days to maturity	Height 1 st branch (cm)	Height 1 st capsule (cm)	Plant height (cm)	Number branches per plant	Number capsules on branches	Number capsules on mainstem	Total number of capsules	Length of capsule zone (cm)	Yield per plant (gm)
GCA (Line)	8	6.45***	1.434*	62.17***	59.77**	34.66	0.494*	41.05	23.1***	142.9***	43.4	4.958***
GCA (Tester)	4	27.64***	1.776**	143.15**	293.58***	124.39**	0.957*	71.75*	9.86	161.08**	145.82**	2.264
SCA	32	1.51*	0.407	19.95	20.48	43.8	0.209	35.54*	11.88**	63.55*	64.72*	1.759**
BR		0.848	1	0.888	0.923	0.489	1	0.409	0.412	0.600	1	-
CGD _{BSH}		0.868	0.468	0.612	0.718	0.408	0.369	0.530	0.603	0.595	0.115	-
CGD _{SSH}		0.736	0.468	0.544	0.663	0.200	0.369	0.217	0.139	0.357	0.115	-

*, **, *** Significant at 5%, 1% and 0.1% Probability level; BR= Baker's ratio; CGDBSH= Coefficient of genetic determination for broad sense heritability; CGDNSH= Coefficient of genetic determination for narrow sense heritability.

Coefficients of determination for narrow sense heritability (CDNSH) were also high for days to flowering, height of first branch, height of first capsule indicating that genetic factors contributed highly for these traits, while environmental factors contributed less (Bernardo, 2010).

4.4.2.4. General combining ability effects for Ngetta

General combining ability effects for yield and other selected yield components in sesame at Ngetta are presented in Table 4.9. For the lines, no significant GCA effect was recorded for height of first branch. This means, there was no additive gene action for this trait at this location. Positive significant effect for height of first capsule was recorded for Local 158//6022 and Sesim 2. This effect is not desirable and it could reduce the number of capsules on the main stem and yet the latter is positively correlated to yield per plant.

Sesim 1 had positive significant ($P<0.05$) effects for plant height. Sesim 1 would be a desirable parent for increasing plant height which is normally positively correlated with seed yield.

Ajimo A1-6//7029 and Sesim 2 had positive significant ($P<0.05$) effects for number of branches. As indicated for plant height, this trait is also positively correlated to other traits such as seed yield, days to flowering and maturity. Thus, these are good parents for improvement of the number of branches per plant in the progenies.

Sesim 1 had positive significant ($P<0.05$) effects for the length of capsule zone. Sesim 1 used as a female parent is desirable for increasing the length of the capsule zone.

For yield per plant, Ajimo A1-6//7029 had high positive significant ($P<0.001$) effects. This parent is then very important in improving the yield of sesame in Uganda if used as a female parent since it has a significant GCA effect and therefore could contribute to the progeny.

Table 4.9: General combining ability effects for various yield components in sesame

Genotypes	Height 1 st branch (cm)	Height 1 st capsule (cm)	Plant height (cm)	Number branches per plant	Number capsules on branches	Number capsules mainstem	Total number capsules on capsules	Length of capsule zone (cm)	Yield per plant (gms)
Lines									
Sesim 1	-1.87	-1.88	5.68*	-0.11	-1.53	0.5	-1.08	5.38*	-0.46
Sesim 2	5.77	4.12*	1.62	0.35*	0.27	-0.83	-0.55	-6.29**	-0.51
Ajimo A1-5	3.48	0.26	2.20	-0.11	0.47	-0.96	-0.54	-0.82	-0.49
Ajimo A1-6/7029	1.55	-3.01	-0.88	0.42*	2.20	0.57	2.72	1.78	1.81**
EM15-1-5	0.53	-2.47	-3.32	-0.25	-1.13	1.04	0.05	-0.15	-0.93
U1-7	0.15	-0.34	1.10	-0.31	0.53	0.97	1.46	2.05	1.13
Adong 4-4	-0.11	0.12	1.14	-0.05	0.27	-0.3	-0.08	0.11	-0.42
Local 158/6022	-5.92	4.52*	-7.94**	0.17	-0.25	-0.56	-0.75	-2.68	-0.32
(Y-1/Local 158)-1-2-1	-3.58	-1.34	0.4	-0.14	-0.8	-0.43	-1.21	0.65	0.19
S.E	2.81	2.11	2.70	0.18	1.33	0.83	1.53	2.36	0.66
Testers									
Local 158	4.42*	6.99***	-2.70	0.13	1.75	-1.73**	0.06	-1.74**	-0.68
SPS1438-1-6-4	-6.04**	-7.86***	-1.55	-0.17	-2.0*	-0.33	-2.38*	5.82**	-0.14
4036-1-10-2	0.42	2.4	2.63	0.16	-0.04	2.34***	2.29*	-1.00	1.64**
Renner 1-3-1-1	0.79	1.03	-0.08	0.05	1.07	-0.07	0.96	-2.22	-0.15
7029-1-2	0.41	-2.56	1.71	-0.17	-0.78	-0.22	-0.93	2.15	-0.67
S.E	2.09	1.58	2.01	0.13	0.99	0.62	1.14	1.76	0.49

*, **, *** Significant at 5%, 1% and 0.1% Probability level.

for the testers, 4036-1-10-2 had highly positive significant effect ($P<0.001$) for number of capsules on main-stem. 4036-1-10-2 also had positive significant effect ($P<0.05$) for total number of capsules and therefore, it is a desirable male parent for increasing the number of capsules on main stem and total number of capsules per plant. SPS1438-1-6-4 had high positive significant effect ($P<0.01$) for length of capsule zone.

Lastly, 4036-1-10-2 had high significant effect ($P<0.01$) on yield per plant. This parent is therefore important for improving the yield of sesame in Uganda as a male parent.

From the results recorded above, it can be seen that among the females used as lines, Ajimo A1-5//7029 and Sesim 1 were the best female lines with traits that included number of branches per plant and yield per plant for the former and plant height and length of capsule zone for the latter. For the testers used as males, SPS1438-1-6-4 recorded four significant effects for decreasing height of first branch, height of first capsule and length of capsule zone. This was followed by 4036-1-10-2 with three significant traits that included increasing the number of capsules on the main stem, total number of capsules and yield per plant.

4.4.2.5 Estimates of specific combining ability effects at Ngetta

Estimates of specific combining ability effects for yield and yield components in sesame at Ngetta are presented in Table 4.10. Sesim 1 x 7029-1-2 and Local 158//6022 x SPS1438-1-6-4 had significant positive effect ($P<0.5$) for height of first branch.

For height of first capsule, significant positive SCA effect were recorded in Local 158//6022 x Local 158 ($P<0.001$), Sesim 2 x 4036-1-10-2 ($P<0.05$) and Sesim 2 x SPS1438-1-6-4 ($P<0.05$). Significant negative effect were recorded in Sesim 2 x 7029-1-2 ($P<0.05$), Local 158//6022 x 4036-1-10-2 ($P<0.01$), Ajimo A1-6//7029 x Local 158 ($P<0.05$), (Y-1//Local 158)-1-2-1 x Local 158 ($P<0.05$).

For plant height, only significant negative effect ($P<0.05$) were recorded in (Y-1//Local 158)-1-2-1 x Local 158, Ajimo A1-6//7029 x 4036-1-10-2, Adong 4-4 x Renner 1-3-1-1. This is not desirable as plant height is positively correlated with yield per plant.

Number of branches per plant had Sesim 1 x Renner 1-3-1-1 recorded significant positive SCA effect ($P < 0.05$) and Adong 4-4 x SPS1438-1-6-4 recorded significant ($P < 0.05$) negative SCA effect. Higher number of branches in Sesim 1 x Renner 1-3-1-1 means more capsules and therefore this contributes positively to yield per plant. The cross Adong 4-4 x SPS1438-1-6-4 would not be good since the capsules on branches and on the main stem would be reduced and so this would consequently reduce yield per plant.

Table 4.10: Estimates of specific combining ability effects for yield and yield components

Cross	Height 1 st branch (cm)	Height 1 st capsule (cm)	Plant height (cm)	Number branches per plant	Number capsules on branches	Number capsules on mainstem	Total number of capsules	Length of capsule zone (cm)	Yield per plant (gms)
1 Sesim 1 x Local 158	-4.79	-0.45	-4.24	-0.27	1.44	-1.14	-2.06	-6.52	0.80
2 Sesim 1 x SPS1438-1-6-4	-4.67	-1.27	2.31	0.37	2.20	1.46	3.71	4.92	0.50
3 Sesim 1 x 4036-1-10-2	-5.79	-5.20	1.43	-0.29	-1.76	1.12	-0.63	8.07	-1.71
4 Sesim 1 x Renner 1-3-1-1	0.50	3.17	3.14	0.82*	-2.13	0.20	2.38	2.29	2.11
5 Sesim 1 x 7029-1-2	14.76*	3.76	-2.65	-0.63	-1.69	-1.65	-3.40	-8.75	-1.70
6 Sesim 2 x Local 158	-0.43	-7.79	-6.18	-0.41	0.32	-1.14	-1.16	-1.20	-1.12
7 Sesim 2 x SPS1438-1-6-4	10.03	10.06*	7.37	0.57	2.74	-0.21	2.51	2.59	0.84
8 Sesim 2 x 4036-1-10-2	1.57	10.47*	8.19	0.24	1.44	2.12	3.84	2.07	0.56
9 Sesim 2 x Renner 1-3-1-1	-2.80	-2.49	-2.10	0.02	-1.34	0.20	2.51	-9.71	-0.12
10 Sesim 2 x 7029-1-2	-0.37	-10.24*	-7.29	-0.43	-3.15	-0.99	-4.26	6.26	-0.16
11 Ajimo A1-5 x Local 158	0.18	-3.26	10.24	0.07	4.78	2.33	7.07*	-0.66	0.27
12 Ajimo A1-5 x SPS1438-1-6-4	8.97	6.26	4.09	0.37	1.20	0.60	1.85	1.12	0.43
13 Ajimo A1-5 x 4036-1-10-2	-0.49	-1.10	-11.09	-0.63	-0.69	-1.41	-3.82	-6.06	0.72
14 Ajimo A1-5 x Renner 1-3-1-1	0.14	1.03	2.92	0.48	-2.87	0.67	-2.16	3.82	-0.93
15 Ajimo A1-5 x 7029-1-2	-7.81	-2.04	-6.17	-0.29	-0.69	-2.18	-2.94	1.79	-0.47
16 Ajimo A1-6//7029 x Local 158	-6.21	-11.99*	4.62	0.20	-2.28	1.47	-0.86	11.74*	1.47
17 Ajimo A1-6//7029 x SPS1438-1-6-4	-0.10	-0.81	-0.83	-0.16	-1.86	0.72	-1.09	-0.15	0.26
18 Ajimo A1-6//7029 x 4036-1-10-2	2.45	1.27	-12.71*	0.17	-0.49	-3.60	-4.09	-15.33**	-1.02
19 Ajimo A1-6//7029 x Renner 1-3-1-1	0.74	2.97	2.70	-0.38	1.06	0.13	1.25	3.22	1.47
20 Ajimo A1-6//7029 x 7029-1-2	3.12	8.56	6.21	0.17	3.58	1.28	4.80	0.52	0.82
21 Adong 4-4 x Local 158	11.81	3.15	10.46	0.20	-1.62	3.33	1.47	2.01	1.73
22 Adong 4-4 x SPS1438-1-6-4	-8.85	-5.68	-6.39	-0.83*	-1.87	-3.07	-5.09	-1.55	-2.48

Cross	regent 1 branch (cm)	regent 1st capsule (cm)	Plant height (cm)	Number branches per plant	capsules on branches	capsules on mainstem	number of capsules	capsule zone (cm)	per plant (gms)
23	Adong 4-4 x 4036-1-10-2	3.14	6.40	7.13	0.17	-0.16	-1.07	-0.07	0.85
24	Adong 4-4 x Renner 1-3-1-1	-5.91	-6.89	-12.56*	-0.05	-1.27	-1.00	0.49	0.70
25	Adong 4-4 x 7029-1-2	-0.19	3.03	1.35	0.51	4.91	1.82	-0.88	-0.81
26	UI-7 x Local 158	5.19	4.67	5.04	0.27	3.05	-0.94	2.14	0.14
27	UI-7 x SPS1438-1-6-4	-0.05	-1.81	1.89	-0.76	-2.87	2.32	1.25	2.40
28	UI-7 x 4036-1-10-2	0.52	-2.07	0.01	0.24	-0.16	-0.33	-0.27	-0.98
29	UI-7 x Renner 1-3-1-1	-2.18	-0.70	-3.58	0.02	1.73	-1.27	-1.05	-0.92
30	UI-7 x 7029-1-2	-3.48	-0.10	-3.37	0.27	-1.75	-0.45	-2.08	-0.64
31	EM15-1-5 x Local 158	4.11	-0.79	1.00	0.00	1.32	-1.67	-0.60	0.40
32	EM15-1-5 x SPS1438-1-6-4	-9.76	-0.60	-10.85	-0.03	-0.60	-0.41	-3.48	0.43
33	EM15-1-5 x 4036-1-10-2	2.78	2.80	4.27	0.31	1.77	0.92	1.34	-1.32
34	EM15-1-5 x Renner 1-3-1-1	6.08	3.17	6.98	-0.25	0.33	1.00	5.23	0.50
35	EM15-1-5 x 7029-1-2	-3.21	1.43	-1.41	-0.03	-2.82	0.15	-2.48	-0.01
36	Local 158//6022 x Local 158	-10.10	27.45***	-7.72	0.20	-3.09	-1.05	-0.11	-0.75
37	Local 158//6022 x SPS1438-1-6-4	13.05*	1.66	4.23	0.09	-0.42	-2.47	-6.36	-1.82
38	Local 158//6022 x 4036-1-10-2	-9.42	-15.60**	-3.25	-0.24	0.29	0.85	4.79	2.30
39	Local 158//6022 x Renner 1-3-1-1	0.88	-7.56	1.76	-0.13	2.51	1.26	1.02	-1.31
40	Local 158//6022 x 7029-1-2	5.59	-5.96	4.97	0.09	0.69	1.41	0.65	1.58
41	(Y-1//Local 158)-1-2-1 x Local 158	0.25	-10.99*	-13.26*	-0.27	-1.62	-1.21	-5.31	0.08
42	(Y-1//Local 158)-1-2-1 x SPS1438-1-6-4	-8.62	-1.81	-1.81	0.37	1.47	1.06	1.65	-0.55
43	(Y-1//Local 158)-1-2-1 x 4036-1-10-2	6.25	3.93	6.01	0.40	1.51	0.73	5.47	0.60
44	(Y-1//Local 158)-1-2-1 x Renner 1-3-1-1	2.55	7.30	0.72	-0.52	-2.27	-1.20	-5.31	-1.51
45	(Y-1//Local 158)-1-2-1 x 7029-1-2	-0.42	1.56	8.33	0.37	0.91	0.61	4.98	1.39
	SE	6.27	4.73	6.04	0.40	2.98	1.85	5.29	1.48

*, **, *** Significant at 5%, 1% and 0.1% Probability level.

4.4.2.6. General and specific combining ability variances at Ngetta

Results of the general (GCA), specific (SCA) combining ability variances for yield and some of its components at Ngetta are presented in Table 4.11. The females (lines) showed significant GCA variances ($P<0.05$) for number of branches per plant only while the testers showed highly significant GCA variances ($P<0.001$) for height of first capsule, number of capsules on main stem and length of capsule zone suggesting that the lines and the testers contributed differently to the variation in those traits. High significant GCA variances ($P<0.01$) were recorded for yield per plant. Highly significant effect for SCA ($P<0.001$) was observed only for height of first capsule suggesting that only this trait contributed for variability for SCA variance.

Baker's ratio was high for the majority of traits suggesting that both the lines and the testers contributed much/highly to the performance of the progeny (Baker, 1978). The coefficient of determination of broad sense (CDBSH) and narrow sense (CDNSH) heritabilities were generally low indicating that environmental factors played a greater role in the phenotype than the genetic component. This could have been caused by drought that set in during physiological maturity thus causing low yield performance (Figure 5.2).

Table 4.11: General (GCA) and specific (SCA) combining ability variances, Baker's ratio and coefficient of genetic determination for yield and its components at Ngetta.

Parameter	D.F	Height 1 st branch (cm)	Height 1 st capsule (cm)	Plant height (cm)	Number branches per plant	Number capsules on branches	Number capsules on mainstem	Total number of capsules	Length of capsule zone (cm)	Yield per plant (gms)
(GCA) Line	8	62.15	36.31	73.27	0.331*	6.138	2.996	8.33	52.59	4.023
(GCA) Tester	4	128.21*	279.02***	43.97	0.237	19.869	19.4***	28.55	150.4***	8.177**
SCA	32	51.97	70.13***	55.7	0.184	6.173	3.127	12.32	33.21	1.914
BR		0.534	0.396	0.299	0.627	1	1	0.754	0.779	1.0
CGD _{BSH}		0.407	0.780	0.429	0.303	0.120	0.342	0.175	0.459	0.320
CGD _{NSH}		0.217	0.309	0.128	0.190	0.120	0.342	0.132	0.358	0.320

*, **, *** Significant at 5%, 1% and 0.1% Probability level; BR= Baker's ratio; CGDBSH= Coefficient of genetic determination for broad sense heritability; CGDNSH= Coefficient of genetic determination for narrow sense heritability.

4.4.2.7. Combined estimates for general combining ability effects for Serere and Ngetta

Combined general combining ability effects are presented in Table 4.12. Among the lines (females), significant ($P < 0.01$) negative effects for height to first branch and first capsule was recorded in Local 158//6022. This parent would be a desirable one since capsules would also be set lower on the stem and possibly more capsules set on the main stem if the plant height is maintained.

For plant height, high positive but non-significant effects was recorded in Sesim 1 and high negative but non-significant effects recorded in Local 158//6022. Sesim 1 would be a desirable parent for increasing plant height and thus improving seed yield per plant as they are positively correlated. Thiagarajan and Ramanathan (1995) reported the predominance of additive gene action in plant height.

Positive significant effect ($P < 0.05$) was recorded only in Local 158//6022 for number of capsules on branches ($P < 0.01$), number of capsules on main stem and total number of capsules per plant ($P < 0.001$). Therefore this parent can be used to increase the number of capsules on the branches, number of capsules on the main stem and the overall total number of capsules per plant thus increasing seed yield per plant indirectly.

Among the tester (males), highly positive significant ($P < 0.001$) GCA effect was recorded in Local 158 and highly negative significant ($P < 0.001$) effects was recorded in SPS1438-1-6-4 for height to first branch and height to first capsule. SPS1438-1-6-4 would then be a desirable parent as a source for increasing height of first branch and height of first capsule on the main stem. Zhao Ying Zhong (1999) reported that additive gene action was important for height to first capsule although non-additive gene action also played a role.

Length of capsule zone had SPS1438-1-6-4 and 4036-1-10-2 with high positive significant ($P < 0.05$) GCA effects and Local 158 had significant negative GCA effect. SPS1438-1-6-4 and 4036-1-10-2 would then be desirable parents for increasing the length of the capsule zone.

Table 4.12: Estimation of combined GCA effects for Serere and Ngetta for various characters in sesame

Lines	Days to flower	Days to maturity	Plant height (cm)	Height 1 st branch (cm)	Height 1 st capsule (cm)	Number branches per plant	Number capsules on branches	Number capsules on mainstem	Total number of capsules	Length of capsule zone (cm)	Yield per plant (gms)
Sesim 1	-0.18	-0.63	3.68	-1.52	-0.55	0.12	-1.32	0.91	-0.78	3.89	-0.07
Sesim 2	0.82*	0.11	-0.80	-0.12	-0.21	0.06	-0.76	-0.62	-2.24	-0.64	-0.56
Ajimo A1-5	0.89*	0.63*	1.16	0.93	5.19**	0.10	1.34	-1.29	-1.38	-3.51	-0.63
Ajimo A1-6/7029	-1.65***	-0.17	-2.26	-0.12	-2.09	-0.41	-1.98	-0.89	-2.11	-0.65	1.10
EM15-1-5	1.22**	0.29	2.82	2.07	-0.27	-0.30	-0.81	1.84	1.24	2.96	-0.32
UI-7	0.63	0.55	0.48	-1.12	-1.08	-0.14	2.08	2.31*	3.96	2.22	-0.44
Adong 4-4	-0.18	0.39	-2.84	-1.12	-1.88	-0.28	-4.38*	-1.22	-6.31*	-0.11	-1.00
Local 158/6022	-1.96***	-0.43	-3.86	-6.42**	-4.82*	0.35	7.16**	4.59***	14.16***	0.97	0.90
(Y-1/Local 158)-1-2-1	0.02	-0.83**	0.46	6.15***	4.72*	0.59**	0.08	-2.35**	-3.71	-4.91	-0.44
S.E	0.39	0.29	2.55	1.84	1.85	0.22	2.06	1.12	2.83	2.65	1.56
Testers											
Local 158	1.37***	0.43	3.57	6.67***	8.21***	0.31	2.83	-0.78	2.69	-4.5*	-0.46
SPS1438-1-6-4	-2.97***	-0.57**	-4.13*	-4.88***	-8.08***	-0.53***	-4.79**	-0.27	-7.24***	4.11*	-0.41
4036-1-10-2	1.10***	0.49*	2.94	-1.14	-0.97	-0.05	0.40	1.84*	3.44	3.96*	0.92
Renner 1-3-1-1	0.77**	-0.08	-3.87*	-0.17	-0.60	0.22	0.71	-0.34	0.47	-3.82	0.04
7029-1-2	-0.12	-0.23	1.90	0.27	2.36	0.08	1.16	-0.53	0.95	-0.23	-0.09
S.E	0.29	0.21	1.90	1.37	1.38	0.16	1.53	0.83	2.11	1.98	-

4.4.2.8. Combined estimates for specific combining ability effects for Serere and Ngetta.

Combined SCA effects are presented in Table 4.13. Significant ($P < 0.01$) negative effects was recorded in Ajimo A1-6//7029 x local 158 for days to flowering and positive significant effect in Local158//6022 x SPS1438-1-6-4. Significant ($P < 0.05$) negative effects was recorded in U1-7 x Renner 1-3-1-1 for days to maturity. Significant ($P < 0.01$) negative effects was recorded in EM15-1-5 x Renner 1-3-1-1 for height of first branch. Positive ($P < 0.05$) significant effects was recorded in adong 4-4 x Local 158 for height of first capsule. Sesim 1 x SPS 1438-1-6-4 had significant ($P < 0.05$) positive effect for plant height. Local158//6022 x SPS1438-1-6-4 had positive significant ($P < 0.05$) for number of branches per plant. For number of capsules on branches, positive significant effect was recorded in Sesim 1 x SPS1438-1-6-4, Ajimo A1-6//7029 x Renner 1-3-1-1 and U1-7 x 7029-1-2. For number of capsules on main stem, significant ($P < 0.05$) positive effects were recorded in Sesim 1 x SPS1438-1-6-4, EM15-1-5 x Renner 1-3-1-1 while significant ($P < 0.05$) negative effect was recorded in Local158//6022 x SPS1438-1-6-4. U1-7 x Renner 1-3-1-1 had significant ($P < 0.05$) negative effect for total number of capsules. Significant ($P < 0.05$) positive effect for length of capsule zone was recorded in Sesim 1 x SPS1438-1-6-4, EM15-1-5 x Renner 1-3-1-1 while negative significant effect ($P < 0.01$) was recorded in Local158//6022 x SPS1438-1-6-4. Thiagarajan and Ramanathan (1995) reported the influence of non-additive gene action was observed for number of branches per plant, number of capsules per plant and seed yield. Azeez and Morakinyo (2014) reported that inclusion of F_1 hybrids showing high SCA and having parents with good GCA into multiple crosses could be a worthwhile approach for tangible improvement of the traits. They also reported that hybrid combinations with high means, favourable SCA and involving at least one of the parents with high GCA would tend to increase the concentration of favourable alleles. They argued that the low x low or low x moderate general combiners exhibiting SCA effects suggested gene dispersion and genetic interaction between favourable alleles contributed by both parents.

Table 4.13: Estimation of combined SCA effects for Serere and Ngetta for various characters in sesame

Cross	Days flower	Days maturity	Height 1 st branch (cm)	Height 1 st capsule (cm)	Plant height (cm)	Number branches per plant	Number capsules on branches	Number capsules on mainstem	Total number of capsules	Length of capsule zone (cm)	Yield per plant (gm)
1 Sesim 1 x Local 158	0.80	-0.49	1.95	1.11	-5.23	0.43	-6.23	-3.03	-8.10	-5.32	1.10
2 Sesim 1 x SPS1438-1-6-4	-1.20	1.18	-3.83	-0.94	13.17*	0.27	10.39*	5.79*	11.8	12.06*	1.15
3 Sesim 1 x 4036-1-10-2	-0.28	-0.88	0.42	-3.05	-4.90	-0.21	-4.14	-2.32	-6.51	-4.13	-1.46
4 Sesim 1 x Renner 1-3-1-1	0.40	0.69	0.12	3.58	4.51	-0.15	3.21	3.19	7.8	2.32	0.33
5 Sesim 1 x 7029-1-2	0.29	-0.49	1.34	-0.71	-7.55	-0.34	-3.23	-3.62	-5.02	-4.94	-1.12
6 Sesim 2 x Local 158	0.80	0.43	-2.78	-5.56	-0.45	-0.17	-0.46	1.50	1.37	5.22	-0.39
7 Sesim 2 x SPS1438-1-6-4	-0.86	-0.24	0.77	1.40	6.25	-0.33	-0.70	1.65	1.97	5.93	0.12
8 Sesim 2 x 4036-1-10-2	-0.28	-0.30	1.35	3.62	-4.42	0.19	-3.37	-4.79	-10.38	-9.26	-0.09
9 Sesim 2 x Renner 1-3-1-1	1.40	-0.06	4.05	1.92	-2.31	-0.08	-2.68	-0.61	-3.07	-2.15	0.34
10 Sesim 2 x 7029-1-2	-1.05	0.16	-3.39	-1.38	0.93	0.40	7.21	3.25	10.11	0.26	0.04
11 Ajimo A1-5 x Local 158	0.06	0.91	-1.83	0.38	8.29	0.45	8.44	3.83	8.83	7.75	0.80
12 Ajimo A1-5 x SPS1438-1-6-4	-0.60	-0.76	1.97	-0.33	-8.31	-0.50	-5.30	-2.01	-7.24	-8.54	-0.24
13 Ajimo A1-5 x 4036-1-10-2	-0.34	-0.15	1.64	0.55	1.62	-0.18	-2.14	0.55	0.43	2.61	0.72
14 Ajimo A1-5 x Renner 1-3-1-1	0.33	0.42	-4.67	1.85	0.43	0.54	4.56	-2.61	0.73	-1.28	-0.61
15 Ajimo A1-5 x 7029-1-2	0.55	-0.43	2.89	-2.45	-2.03	-0.31	-5.55	0.25	-2.75	-0.54	-0.66
16 Ajimo A1-6/7029 x Local 158	-3.08**	-0.62	-3.78	-5.36	-3.59	-0.37	-3.24	0.10	-3.77	0.22	-0.94
17 Ajimo A1-6/7029 x SPS1438-1-6-4	0.61	-0.29	-0.90	-2.73	0.11	-0.20	-0.28	1.69	1.84	2.59	0.13
18 Ajimo A1-6/7029 x 4036-1-10-2	1.19	-0.12	3.02	2.15	-4.35	0.32	-1.47	-2.19	-4.18	-8.26	-0.87
19 Ajimo A1-6/7029 x Renner 1-3-1-1	0.19	0.22	-0.62	1.79	8.15	0.38	9.22*	1.66	11.46	5.86	1.56

Cross	Days flower	Days maturity	Height 1 st branch (cm)	Height 1 st capsule (cm)	Plant height (cm)	Number branches per plant	Number capsules on branches	Number capsules on mainstem	Total number of capsules	Length of capsule zone (cm)	Yield per plant (gms)
20	1.09	0.70	2.28	4.15	-0.31	-0.14	-4.23	-1.15	-5.35	-0.41	0.12
21	1.46	0.15	0.89	9.78*	4.69	0.49	4.83	-1.90	3.10	-2.32	-0.05
22	-0.20	-0.51	-3.57	-2.26	3.39	-0.33	-2.88	3.25	2.36	6.73	0.97
23	0.73	0.42	3.35	3.95	0.02	-0.15	-2.41	-1.86	-5.64	-2.13	-0.61
24	-0.94	0.45	3.72	-6.09	-7.47	-0.42	-3.05	-1.67	-5.67	-7.02	0.18
25	-1.05	-0.52	-4.39	-5.38	-0.63	0.40	3.50	2.18	5.85	4.73	-0.49
26	0.65	-0.00	-2.11	-2.36	-5.03	0.03	3.04	-2.10	0.84	-1.98	-0.86
27	-1.34	0.33	-0.90	-4.74	5.37	-0.13	-5.68	3.06	0.43	9.73	-1.39
28	0.29	0.51	1.69	3.15	2.90	0.06	-0.88	0.61	0.42	0.87	-0.31
29	0.59	-1.49*	4.38	3.45	-8.59	-0.22	-8.19	-4.54	-13.93*	-12.68*	-1.64
30	-0.19	0.66	-3.06	0.49	5.35	0.26	11.71**	2.98	12.24	4.06	1.43
31	0.73	0.25	8.03*	4.50	-2.67	0.19	-2.41	-1.96	-3.45	-0.39	0.16
32	0.07	-0.07	-0.42	-2.54	-10.97	0.03	-1.79	-4.81	-8.52	-9.01	-1.77
33	0.33	0.52	-3.90	-2.95	0.96	0.42	6.88	3.06	11.23	1.49	0.76
34	-0.34	0.05	-11.47**	-6.02	5.07	-0.39	3.38	5.26*	9.44	14.25*	1.32
35	-0.78	-0.76	7.75	7.02	7.61	-0.25	-6.07	-1.55	-8.70	1.66	-0.49
36	2.92**	0.30	5.40	7.34	-9.99	1.05*	-2.42	-5.22*	-6.11	-16.68**	-1.51
37	-1.50	-0.42	-3.00	-6.77	1.24	-0.44	6.05	4.00	7.88	8.13	1.09
38	-0.82	0.15	-2.31	-0.80	4.46	-0.05	-1.92	1.52	1.19	4.57	-0.04
39	-0.60	-0.04	-0.09	0.24	4.29	-0.56	-1.71	-0.30	-2.96	3.98	0.46
40	-1.41	-0.63	-0.38	-2.49	3.99	-1.04*	-3.96	3.56	1.17	4.82	0.18

Cross	Days flower	Days maturity	Height 1 st branch (cm)	Height 1 st capsule (cm)	Plant height (cm)	Number branches per plant	Number capsules on branches	Number capsules on mainstem	Total number of capsules	Length of capsule zone (cm)	Yield per plant (gm)
41 (Y-1//Local 158)-1-2-1 x SPS1438-1-6-4	0.60	0.05	1.50	4.80	0.99	0.14	8.66	-3.28	3.44	-2.80	-0.23
42 (Y-1//Local 158)-1-2-1 x 4036-1-10-2	-0.14	0.32	-4.58	-0.65	6.92	-0.01	1.47	2.94	6.76	10.67	0.76
43 (Y-1//Local 158)-1-2-1 x Renner 1-3-1-1	-0.8-	-0.44	6.79	0.32	-4.27	0.38	-4.54	-1.20	-7.94	-3.88	-1.43
44 (Y-1//Local 158)-1-2-1 x 7029-1-2	-0.14	0.32	-3.33	-1.98	-7.63	0.53	-1.63	-2.02	-3.42	-8.81	0.73
SE	0.87	0.64	4.12	4.14	5.69	0.48	4.62	2.50	6.33	5.93	1.33

4.4.2.9. Combined analysis of variance (ANOVA) across locations

The results of ANOVA across locations are presented in Table 4.14. Highly significant ($P < 0.001$ and $P < 0.01$) differences for all the traits across locations were recorded. Therefore, locations had effect on all the traits studied. Significant differences among males were recorded for height to first branch ($P < 0.001$) and number of capsules on main stem ($P < 0.01$). Female x male interaction was significant only for height to first branch ($P < 0.5$) and height to first capsule ($P < 0.01$). Solanki and Gupta (2001) recorded significant variance for line x tester interaction for all the characters. Ram (1995) reported that where there was high variance for line x tester, this indicated high specific combining ability. Saravanan and Nadarajan (2003) using diallel method showed that variances due to general combining ability (GCA) and specific combining ability (SCA) were significant for all the characters studied and GCA variances were greater than the SCA variance for eight traits viz. plant height, number of primary branches per plant indicating the preponderance of additive gene action for these traits. Yamanura and Nadaf (2009) recorded the mean square due to GCA as well as SCA to be significant for all characters meaning that the significance of GCA (variances due to lines and testers) and SCA (variances due to line x testers) implied that both additive and non-additive types of variation were available for all the characters. They also reported that additive genes were more important than the dominant genes because variance due to GCA was higher than that of SCA.

Table 4.14: Mean square variances combined across locations of Serere and Ngetta

Source	d.f	Height 1 st branch (cm)	Height 1 st capsule (cm)	Plant height (cm)	Number branches per plant	Number capsules on mainstem	Total number of capsules	Length of capsule zone (cm)	Yield per plant (gms)
Location	1	1380.06***	214.63**	1895.35***	18.00***	2231.81***	8513.96***	1366.63***	719.19***
Female	8	64.56	58.14	61.42	0.35	17.68	81.55	61.86	4.82
Male	4	292.08***	398.72	106.92	0.83	26.21**	154.02	248.71	7.16
FxM	31	46.94*	43.3**	49.49	0.22	8.65	38.21	59.95	1.16
LxF	8	47.65	29.25	20.39	0.46*	8.23	69.29	30.58	2.43
LxM	4	5.6	67.97**	48.54	0.34	1.05	36.29	40.15	3.85
LxFxM	31	22.12	15.39	48.97	0.18	6.414	37.43	39.03	2.08
Total	87	64.48	52.87	71.5	0.48	34.66	147.43	72.76	10.59
Pooled error	85.5	28.31	19.86	34.58	0.20	4.87	25.89	31.23	1.77

*, **, *** Significant at 5%, 1% and 0.1% Probability level.

4.4.2.10. Combined general and specific combining ability variances at Serere and Ngetta

Combined general (GCA) and specific (SCA) combining ability variances for yield and its components at both Serere and Ngetta are presented in Table 4.15. GCA variances for line were significantly positive for days to flowering ($P<0.01$), days to maturity ($P<0.01$), height to first branch ($P<0.001$), height to first capsule ($P<0.01$), number of branches per plant ($P<0.05$), number of capsules on main stem ($P<0.001$) and total number of capsules ($P<0.001$). GCA variances for testers were significantly positive for days to flowering ($P<0.001$), days to maturity ($P<0.01$), height to first branch ($P<0.001$), height to first capsule ($P<0.001$), number of branches per plant ($P<0.01$), number of capsules on branches ($P<0.05$), total number of capsules ($P<0.001$) and length of capsule zone ($P<0.01$). SCA was only positively significant for days to flowering ($P<0.01$), number of capsules on branches ($P<0.05$), number of capsules on main-stem ($P<0.05$) and total number of capsules ($P<0.05$). Negative significant ($P<0.05$) effects were recorded for length of capsule zone. Baker's ratio was high for most of the characters and medium for plant height, number of capsules on branches and number of capsules on main-stem. Coefficient of determination for broad sense heritability were high for days to flowering, height to first branch, height to first capsule, number of capsules on branches, number of capsules on main-stem and total number of capsules. The coefficients of determination for narrow sense heritability were high for days to flowering, height to first branch and height to first capsule.

Saravanan *et al.* (2000) reported that the SCA variance for seed yield per plant was higher than the GCA variance. Thirugnana Kumar *et al.* (2006) while studying combining ability under normal and temporarily flooded conditions reported that GCA and SCA variances were significant for all the characters in both the environments they used except 1000 seed weight under normal conditions. They suggested that both additive and non-additive genetic components play an important role in the inheritance of these characters.

Table 4.15: Combined General (GCA) and specific (SCA) combining ability variances and gene effects for yield and its components at Serere and Ngetta.

Parameters	D.F	Days to flower	Days to maturity	Plant height (cm)	Height 1 st branch (cm)	Height 1 st capsule (cm)	Number branches per plant	Number capsules on branches	Number capsules on mainstem	Total number of capsules	Length of capsule zone (cm)	Yield per plant (gms)
(GCA) Line	8	1.17**	0.21**	0.48	9.46***	8.93**	0.05*	4.13	3.52***	21.53***	-12.70	5.30***
(GCA) Tester	4	3.06**	0.15**	10.45	14.34***	31.42***	0.08**	5.73*	0.41	13.76***	4.58**	0.05
SCA	32	0.76**	-0.01	11.41	2.99	3.36	-0.02	14.24*	5.61*	23.52*	-40.78*	0.01**
BR		0.85	1	0.49	0.89	0.92	1	0.41	0.41	0.60	1	-
CDBSH		0.87	0.47	0.41	0.61	0.72	0.37	0.53	0.60	0.59	0.12	-
CDNSH		0.74	0.47	0.20	0.54	0.66	0.37	0.22	0.14	0.36	0.12	-

*, **, *** Significant at 5%, 1% and 0.1% Probability level.

4.5 Conclusions

The results suggested that both additive and non-additive gene actions played a role in these traits. The variances for the males were higher than the variances of the females for all characters suggesting greater variability among males than females. The introductions (males) therefore could play a great role in improving the traits of the local genotypes (females). The different crosses recorded different gene actions of additive, dominance and different epistasis types.

CHAPTER FIVE

ANALYSIS OF GENOTYPE BY ENVIRONMENT INTERACTION IN SESAME

(*Sesamum indicum* L.) CULTIVARS IN UGANDA

5.1 Abstract

The improvement of new genotypes with the desired yield stability and performance in different environments is an important issue in breeding programs. In order to identify high yielding and stable sesame genotypes across environments, field experiments were conducted with 16 genotypes for four seasons (2011-2013) at three locations, viz. Serere (latitude 1°31'N; longitude 33°27'E; and altitude 1,128 masl), Kaberamaido (latitude 1°44'N; longitude 33°09'E; altitude 1,080 masl) and Ngetta (latitude 2°17'N; longitude 32°56'E; altitude 1,189 masl). ANOVA, AMMI and GGE were applied to analyse the data. AMMI analysis of variance for seed yield (kg/ha) showed that all the sources of variations that included treatments, genotypes, environments, blocks, interactions, IPCA1 and IPCA 2 were highly significant ($P < 0.001$). The combined analysis of variance indicated that season, season x location, genotype and location x genotype had significant ($P < 0.001$) variation. The GGE biplot suggested the existence of only one sesame mega-environment with genotype G9 winning in that mega-environment followed by G1. The mega-environment had environments K2011B, K2012A, K2012B, N2012B and K2013B. The vertex genotypes which indicated that they were the most responsive in their respective environments were G2, G3, G8, G9, G12 and G14. Genotypes were categorized into stable and high yielding, stable but poor yielding, unstable but good yielding and unstable and poor yielding. Genotypes G2 and G12 performed poorly in poor environments. Environment K2013B was the most discriminating environment. According to the ideal-genotype biplot, genotype G9 was the best genotype and Kaberamaido was the nearest to ideal environment. G9 was officially released as Sesim 3 variety for commercial production because of its high yield, stability, tolerance to pests and high oil content.

Key words: AMMI, GGE biplot, mega-environment, principal component analysis, stability

5.2 Introduction

In variety selection experimentation, many genotypes are normally tested over a wide range of environments. Plant breeders perform multi-environmental trials (MET) to evaluate new improved genotypes across test environments before a specific genotype is released for production (Karimizadeh *et al.*, 2013). In such experiments, genotype x environment (GE)

interaction is commonly evaluated (Yan *et al.*; 2007). In most of the plant breeding programs, GE interaction effects are of special interest for identifying the most stable genotypes, mega-environments and adaptation targets (Sabaghnia *et al.* 2013).

Understanding the structure and nature of genotype x environment interaction (GEI) is important in plant breeding programs because a significant GEI can seriously impair efforts in selecting superior genotypes relative to new crop introductions and cultivar development programs. Information on the structure and nature of GEI is particularly useful to breeders because it can help determine if they need to develop cultivars for all environments of interest or if they should develop specific cultivars for specific target environments.

Multivariate techniques like the additive main effects and multiplicative interaction (AMMI) procedure with prediction assessment can be a powerful tool in analyzing multiplication trials (Gauch and Zobel 1988). The AMMI model combines regular analysis of variance (ANOVA) for additive effects with Principal Component Analysis (PCA) for multiplicative structure within the interaction. A modification of the conventional AMMI analysis proposed by Yan *et al.* (2000) called GGE biplot (Genotype and Genotype-Environment Interaction) has been used to study the GE interaction. The GGE analysis groups together the GE interaction multiplication effect and the genotype effect, which is an additive effect in the AMMI analysis, and analyses these effects by principal components. The objective of this study was to establish the stability and yield performance of sixteen sesame genotypes.

5.3 Materials and Methods

5.3.1 Materials

Sixteen genotypes including the local selected breeding lines and introduced accessions (Table 5.1) selected for better yield potential and other yield characters among genotypes at National Semi-Arid Resources Research Institute (NaSARRI). Serere were evaluated in three locations of Serere (latitude 1°31'N; longitude 33°27'E; and altitude 1,139 masl) , Kaberamaido (latitude 1°44'N; longitude 33°09'E; altitude 1,080 masl) and Ngetta (latitude 2°17'N; longitude 32°56'E; altitude 1,189 masl) for four seasons. The experiments were done in 2011B, 2012A, 2012B and 2013B seasons. The first rainy season planting started about mid March and got harvested in July, while the second season's planting started about mid July and got harvested about December depending on the onset of rains.

5.3.2 Experimental Design

A randomized complete block design with 3 replications was used for all the genotypes across locations and seasons. Each entry was planted in eight 4-m rows, with spacing of 30 cm between rows and continuous sowing within a row. Thinning within the rows was done to approximately 10 cm between plants after first weeding. Standard agronomic practices were followed in order to produce good results. The six middle rows were harvested and used to estimate yield per plot and converted to yield per hectare. Insecticides and fungicides were applied where necessary in order to control insect pests and fungal diseases respectively.

Table 5.1: Genotypes, origin and their major identifiable characteristics

Code name	Genotype	Origin	Yield	Resistance to Fusarium wilt disease	Resistance to Cylindrosporium leaf spot	Resistance to gall midge	Hairiness on capsules	Branching habit	Plant height	Number of capsules per plant	Days to maturity
G1	(Ajimo A1-6/7029)-1-1	A cross between a selected Ugandan land race (Ajimo A1-6) and a Thai accession (7029)	High	Resistant	Resistant	Highly Resistant	Hairy	High	Tall	Many	>85
G2	(Ajimo A1-6/7029)-1-9	A cross between a selected Ugandan land race (Ajimo A1-6) and a Thai accession (7029)	Low	Susceptible	Susceptible	Resistant	Hairy	less	Short	Few	Less than 75
G3	(Local 158/6022)-1-2-1	A cross between an Ethiopian accession (Local 158) and a Thai accession (6022)	High	Resistant	Resistant	Highly resistant	Hairy	High	Tall	Many	>85
G4	(Sesim2/5181)-2-1	A cross between a released commercial variety (Sesim 2) and a Thai accession (5181)	High	Resistant	Medium resistant	Highly resistant	Hairy	Medium	Tall	Many	>85
G5	AD-1-1-1	Selected Ugandan land race	Medium	Resistant	Resistant	Medium resistant	Smooth	High	Tall	Many	>85
G6	Adong 4-4	Selected Ugandan land race	Medium	Resistant	Resistant	Medium resistant	Smooth	High	Tall	Many	>85
G7	EM15-1-5	Selected Ugandan land race	Low	Resistant	Medium resistant	Medium resistant	Light hairy	High	Tall	Medium	>85
G8	EM15-3-2	Selected Ugandan land race	Medium	Resistant	Medium resistant	Medium resistant	smooth	High	Tall	Many	>85
G9	Local 158-1	Ethiopian selection	High	Medium resistant	Medium resistant	Highly resistant	Hairy	Medium	Tall	Many	>80

Code name	Genotype	Origin	Yield	Resistance to Fusarium wilt disease	Resistance to Cylindrosporium leaf spot	Resistance to gall midge	Hairiness on capsules	Branching habit	Plant height	Number of capsules per plant	Days to maturity
G10	Local 158-5	Ethiopian selection	High	Medium resistant	Medium resistant	Highly resistant	Hairy	Medium	Tall	Many	>80
G11	Renner 1-3-1-14	USA selection	Low	Resistant	Medium resistant	Medium resistant	smooth	Medium	Medium	Few	>80
G12	Renner 1-3-1-16	USA selection	Low	Resistant	susceptible	Medium resistant	smooth	Medium	Short	Few	>80
G13	Renner 1-3-1-17	USA selection	Medium	Resistant	Medium resistant	Medium resistant	smooth	Medium	Medium	Medium	>80
G14	Renner 1-3-1-17-1	USA selection	High	Resistant	Medium resistant	Medium resistant	smooth	Medium	Medium	Medium	>80
G15	Sesim 1	Ugandan commercial variety	High	Resistant	Resistant	Low resistant	smooth	High	Tall	Many	>85
G16	Sesim 2	Ugandan commercial variety	High	Resistant	Medium resistant	Medium resistant	smooth	High	Tall	Many	>85

5.3.3 Statistical analysis

Analysis of variance (ANOVA) for yield was carried out for individual locations, seasons and for combined analysis across locations. Statistical procedures applied were Principal Component Analysis (PCA), additive main and multiplicative interaction (AMMI), Genotype and genotype by environment biplot analysis (GGE). For a simple analysis of variance of a randomized complete block design, the model: $Y_{ijk} = \mu + G_i + E_j + GE_{ij} + B_{ij} + \varepsilon_{ijk}$ was applied where μ is the mean, G_i is the effect of the i^{th} genotype, E_j is the effect of the j^{th} environment, GE_{ij} is the interaction of the i^{th} genotype with the j^{th} environment, B_{ij} is the effect of the k^{th} replication in the j^{th} environment, and ε_{ijk} is the random error.

Genotype-focused scaling was used in visualizing for genotypic comparison, with environment-focused scaling for environmental comparison. The symmetric scaling was preferred in visualizing the “which-won-where” pattern of MEYTs yield data (Yan, 2002). The biplots were constructed using GenStat 14th Edition (Payne *et al.*, 2010).

The additive effect and Multiplicative Interaction (AMMI) and GGE procedures for estimating G x E interaction were used. The combined analysis was performed and the means served as basis

for the AMMI analysis, considering the following model: $Y_{ij} = \mu + G_i + E_j + \sum_{k=1}^g \lambda_k \gamma_{ik} \alpha_{jk} + \rho_{ij} + \varepsilon_{ij}$. Where: Y_{ij} is the mean response of genotype i in environment j ; μ is the overall mean; G_i is the genotype effect; E_j is the environment effect; GE_{ij} is the multiplicative component (GE interaction effect) modeled by $\sum_{k=1}^g \lambda_k \gamma_{ik} \alpha_{jk} + \rho_{ij} + \varepsilon_{ij}$, where λ_k is the k^{th} singular of the matrix of original interactions (GE); γ_{ik} is the element corresponding to the i^{th} genotype on the k^{th} singular vector of the GE matrix column; α_{jk} is the element corresponding to the j^{th} environment on k^{th} singular vector of the GE matrix row; ρ_{ij} is the noise associated with expression not explained by the principal components; and ε_{ij} is the associated error.

A GGE biplot was constructed by plotting the first principal components (PC1) against their respective scores for the second principal component (PC2) that results from singular value dimension (SVD) of environment-centred or environment-standardized.

5.4 Results and Discussion

5.4.1 Performance of the genotypes

Results of genotypes performance at Serere are presented in Table 5.2.

Table 5.2: Performance of genotypes at Serere by season and across seasons in kg/ha.

Code no.	Genotype	Serere 2011B	Serere 2012A	Serere 2012B	Serere 2013B	Serere across seasons	Rank
G1	(Ajimo A1-6//7029)-1-1	186	486	702	288	416	6
G2	(Ajimo A1-6//7029)-1-9	60	130	664	381	309	16
G3	(Local 158//6022)-1-2-1	167	266	798	372	401	9
G4	(Sesim2//5181)-2-2-1	190	531	774	292	447	2
G5	AD-1-1-1	195	417	589	370	393	10
G6	Adong 4-4	260	296	835	307	425	4
G7	EM15-1-5	218	293	608	262	345	13
G8	EM15-3-2	132	286	757	323	375	11
G9	Local 158-1	224	604	660	356	461	1
G10	Local 158-5	146	516	691	326	420	5
G11	Renner 1-3-1-14	108	417	815	313	413	7
G12	Renner 1-3-1-16	163	303	552	334	338	15
G13	Renner 1-3-1-17	174	254	900	305	408	8
G14	Renner 1-3-1-17-1	128	407	595	307	359	12
G15	Sesim 1	288	405	755	277	431	3
G16	Sesim 2	101	266	613	392	343	14
	Mean	171	357	707	325	393	
	CV%	24.6	24.6	26.1	34.1		
	LSD	74.5	169	308	185		
	Prob.	0.001	0.001	ns	0.97ns		

At Ngetta, (Table 5.3), the highest yielding genotype was G14 (Renner 1-3-1-17-1) with 571 kgha across seasons although genotype G9 (Local 158-1) had the highest yield of 913 kgha for season 2011B.

Table 5.3: Performance of genotypes for Ngetta by season and across seasons in kg/ha

Cod e no.	Genotype	Ngetta 2011B	Ngetta 2012A	Ngetta 2012B	Ngetta 2013B	Ngetta across seasons	Rank
G1	(Ajimo A1-6//7029)-1-1	700	218	798	546	566	2
G2	(Ajimo A1-6//7029)-1-9	398	247	196	511	338	16
G3	(Local 158//6022)-1-2-1	652	223	486	642	501	8
G4	(Sesim2//5181)-2-2-1	640	196	634	674	536	4
G5	AD-1-1-1	596	196	526	763	520	6
G6	Adong 4-4	716	193	382	608	477	11
G7	EM15-1-5	585	219	590	463	464	12
G8	EM15-3-2	595	209	294	513	403	15
G9	Local 158-1	913	192	487	534	532	5
G10	Local 158-5	431	228	471	647	444	13
G11	Renner 1-3-1-14	649	234	385	442	428	14
G12	Renner 1-3-1-16	897	161	453	530	510	7
G13	Renner 1-3-1-17	844	205	698	418	541	3
G14	Renner 1-3-1-17-1	761	217	748	556	571	1
G15	Sesim 1	711	181	465	531	472	10
G16	Sesim 2	817	235	402	469	481	9
	Mean	681	210	501	553	477	
	CV%	22.8	28.2	45	28		
	SE	155.6	59	221	157		
	Lsd	260	98.5	381	266		
	Prob.	0.01**	0.95ns	0.185ns	0.44ns		

Season 2011B had the highest mean yield of 681 kgha and also recorded the only significant genotype difference ($P < 0.01$) among genotypes at Ngetta. The performance of 2011B and 2013B were fairly high. There was very high coefficient of variability in 2012B showing that other environmental factors played a great role on the performance of the genotypes in the location. Genotype G14 (Renner 1-3-1-17-1), G1 (Ajimo A1-6//7029)-1-1, and G13 (Renner 1-3-1-17) performed well across seasons although generally yields in 2012A were very poor with average mean yields of 210 kg/ha. The genotypes performed similarly in 2012A, 2012B and

The results showed that genotype G9 (Local 158-1) had the highest yield across seasons with 461 kg/ha followed by G4 (Sesim2//5181)-2-2-1 with 447 kg/ha showing that they were the best genotypes at this location. Genotype with the lowest yield was G2 (Ajimo A1-6//7029)-1-9 with 309 kg/ha implying that it was not suitable for this environment. The highest yield recorded at Serere was by genotype G13 Renner (1-3-1-17) in 2012 with 900 kg/ha. Significant difference ($P < 0.01$) in yield performance among genotypes were recorded during 2011B and 2012A. G15 (Sesim 1) recorded the best performance in seed yield per hectare in 2011 followed by G6 (Adong 4-4) and G9 (Local 158-1) with 288, 260 and 224 kg/ha respectively. Due to good rainfall and timely planting in 2012B (Figure 5.1), very good overall mean yield of 700 kg/ha was recorded. Genotype G13 (Renner 1-3-1-17) recorded the best yield of 900 kg/ha followed by Adong 4-4 with 835 kg/ha implying that these genotypes interacted positively with this season. Generally, G9 (Local 158-1), G4 (Sesim2//5181-2-2-1), G15 (Sesim 1) and G6 (adong 4-4) performed well as they were fairly stable across seasons. The released variety G16 (Sesim 2) performed badly especially during 2011B but another released variety, G15 (Sesim 1) did well ranking in the third position.

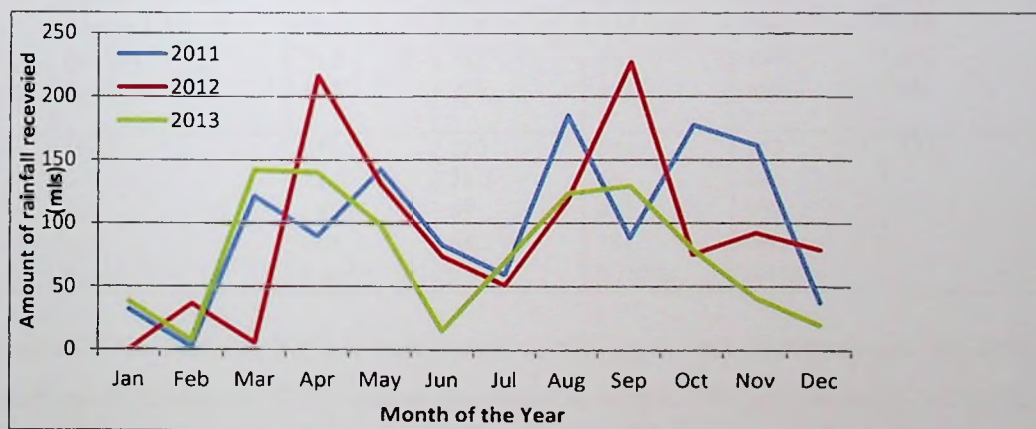


Figure 5.1: Amount of rainfall (mls) at Serere from 2011-2013

At Ngetta, (Table 5.3), the highest yielding genotype was G14 (Renner 1-3-1-17-1) with 571 kgha across seasons although genotype G9 (Local 158-1) had the highest yield of 913 kgha for season 2011B.

Table 5.3: Performance of genotypes for Ngetta by season and across seasons in kg/ha

Cod e no.	Genotype	Ngetta 2011B	Ngetta 2012A	Ngetta 2012B	Ngetta 2013B	Ngetta across seasons	Rank
G1	(Ajimo A1-6//7029)-1-1	700	218	798	546	566	2
G2	(Ajimo A1-6//7029)-1-9	398	247	196	511	338	16
G3	(Local 158//6022)-1-2-1	652	223	486	642	501	8
G4	(Sesim2//5181)-2-2-1	640	196	634	674	536	4
G5	AD-1-1-1	596	196	526	763	520	6
G6	Adong 4-4	716	193	382	608	477	11
G7	EM15-1-5	585	219	590	463	464	12
G8	EM15-3-2	595	209	294	513	403	15
G9	Local 158-1	913	192	487	534	532	5
G10	Local 158-5	431	228	471	647	444	13
G11	Renner 1-3-1-14	649	234	385	442	428	14
G12	Renner 1-3-1-16	897	161	453	530	510	7
G13	Renner 1-3-1-17	844	205	698	418	541	3
G14	Renner 1-3-1-17-1	761	217	748	556	571	1
G15	Sesim 1	711	181	465	531	472	10
G16	Sesim 2	817	235	402	469	481	9
	Mean	681	210	501	553	477	
	CV%	22.8	28.2	45	28		
	SE	155.6	59	221	157		
	Lsd	260	98.5	381	266		
	Prob.	0.01**	0.95ns	0.185ns	0.44ns		

Season 2011B had the highest mean yield of 681 kgha and also recorded the only significant genotype difference ($P < 0.01$) among genotypes at Ngetta. The performance of 2011B and 2013B were fairly high. There was very high coefficient of variability in 2012B showing that other environmental factors played a great role on the performance of the genotypes in the location. Genotype G14 (Renner 1-3-1-17-1), G1 (Ajimo A1-6//7029)-1-1, and G13 (Renner 1-3-1-17) performed well across seasons although generally yields in 2012A were very poor with average mean yields of 210 kg/ha. The genotypes performed similarly in 2012A, 2012B and

2013B as there was no significant difference whereas during 2011B, high significant difference among genotypes was recorded suggesting that in 2011B, there were changes in the rainfall pattern (Figure 5.2) and genotypes had to interact differently with the season compared to the other above mentioned seasons.

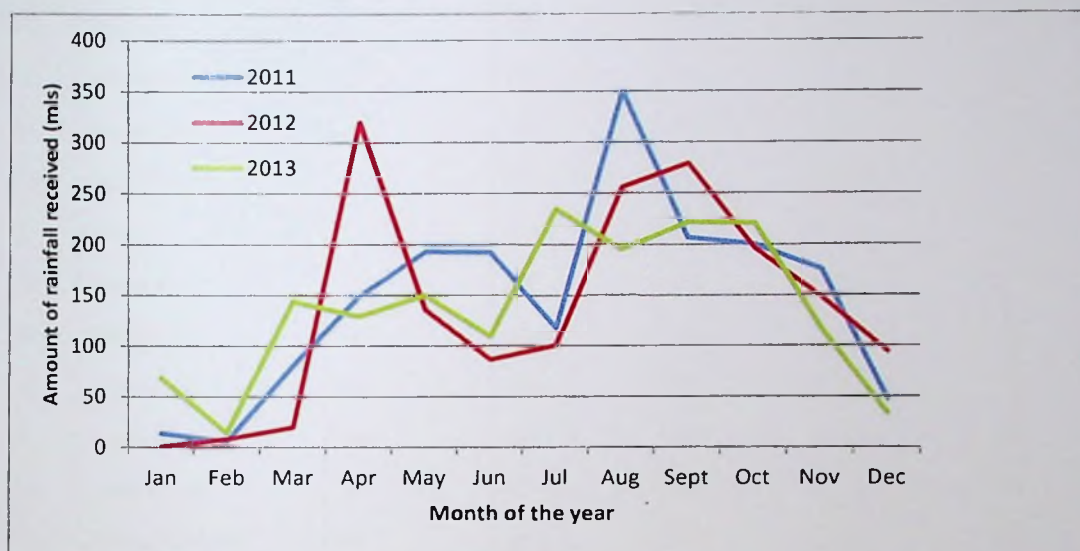


Figure5.2: Amount of rainfall (mls) at Ngetta 2011-2013

Results for Kaberamaido is shown in Table 5.4). Genotype G9 (Local 158-1) was the best yielder across seasons with 700 kg/ha followed by genotype G1(Ajimo A1-6//7029)-1-1 with 587 kg/ha. Season 2013B was the best season with the highest mean yield of 638 kg/ha. Genotype G9 (Local 158-1) was the best yielder in 2012B and 2013B with 865 kg/ha⁻¹ and 963 kg/ha respectively. Genotype G2 (Ajimo A1-6//7029)-1-9 had the poorest mean yield across the location with 234 kg/ha. Highsignificant difference ($P<0.01$) among genotypes was recorded in 2011B and 2013B, and significant difference ($P<0.05$) in 2012B in genotype performance. Highly significant difference among genotypes in 2011B and 2013B showed that the genotypes performed differently during these seasons. The average mean yields for 2011B was very low (192 kg/ha) due to late planting which coincided with decrease in rainfall. This was due to less rainfall during the growing season. G9 (Local 158-1), G1 (Ajimo A1-6//7029-1-1), G3 Local 158//6022-1-2-1), G16 (Sesim 2), G4 (Sesim2//5181-2-2-1) and G15 (Sesim 1) did fairly well due to their stability.

Table 5.4: Performance of genotypes for Kaberamaido by season and across seasons in kg/ha

Code no.	Genotype	Kaberamaido 2011B	Kaberamaido 2012A	Kaberamaido 2012B	Kaberamaido 2013B	Kaberamaido across seasons	Rank
G1	(Ajimo A1-6//7029)-1-1	178	486	779	906	587	2
G2	(Ajimo A1-6//7029)-1-9	19	347	454	117	234	16
G3	(Local 158//6022)-1-2-1	201	579	639	833	563	3
G4	(Sesim2//5181)-2-2-1	220	718	628	556	531	5
G5	AD-1-1-1	255	440	562	701	490	8
G6	Adong 4-4	229	509	560	733	508	7
G7	EM15-1-5	185	463	604	670	481	9
G8	EM15-3-2	293	486	381	721	470	10
G9	Local 158-1	301	671	865	963	700	1
G10	Local 158-5	117	625	486	618	462	12
G11	Renner 1-3-1-14	96	301	455	319	293	14
G12	Renner 1-3-1-16	108	463	382	194	287	15
G13	Renner 1-3-1-17	109	671	400	699	470	10
G14	Renner 1-3-1-17-1	151	579	444	643	454	13
G15	Sesim 1	347	555	405	793	525	6
G16	Sesim 2	263	555	597	747	541	4
	Mean	192	528	540	638		
	CV%	38	32	32	24		
	SE	74	172	174	153		
	SED	61.9	141	142	131		
	Lsd	126.9	287	290	269		
	Prob.	0.001***	0.24ns	0.051*	0.001***		

6.4.2 Combined mean performance analysis across locations

The overall mean yield performance across locations over seasons is presented in Table 5.5.

Table 5.5: Mean yield performance (kg/ha) and ranking of each genotype for each location over four seasons.

Code no.	Genotype	NaSARRI	Kabera maído	Ngetta	Overall mean	Overall rank
G1	(Ajimo A1-6//7029)-1-1	416	587	566	523	2
G2	(Ajimo A1-6//7029)-1-9	309	234	338	294	16
G3	(Local 158//6022)-1-2-1	401	563	501	488	4
G4	(Sesim2//5181)-2-2-1	447	531	536	505	3
G5	AD-1-1-1	393	490	501	461	8
G6	Adong 4-4	425	508	477	470	7
G7	EM15-1-5	345	481	464	430	12
G8	EM15-3-2	375	470	403	416	13
G9	Local 158-1	461	700	532	564	1
G10	Local 158-5	420	462	444	442	11
G11	Renner 1-3-1-14	413	293	428	378	14
G12	Renner 1-3-1-16	338	287	510	378	14
G13	Renner 1-3-1-17	408	470	541	473	6
G14	Renner 1-3-1-17-1	359	454	571	461	8
G15	Sesim 1	431	525	472	476	5
G16	Sesim 2	343	541	481	455	10
Mean		393	475	485	451	
Cv%		19.7	24.8	24		
Prob.		0.218ns	0.001***	0.38ns		

Genotype G9 (Local 158-1) had the best yield of 564 kg/ha followed by G1 (Ajimo A1-6//7029)-1-1 with 523 kg/ha. G2 (Ajimo A1-6//7029)-1-9 was the poorest yielding genotype across locations. Genotype G2 is an early maturing genotype but is susceptible to sesame Fusarium wilt

which tends to reduce its yield in some seasons. Other poor yielding genotypes were G11 (Renner 1-3-1-14) and G12 (Renner 1-3-1-16). The Renner genotypes originated from USA and they have low adaptability although selections G13 (Renner 1-3-1-17) and G14 (Renner 1-3-1-17-1) performed better. The Renner series are pure lines selections made from the original Renner line introduction. The local selected lines are adapted to the local conditions as they have withstood selection pressure within the environment over the years. Local 158-1, Local 158-5 and Sesim2//5181 are hairy genotypes and they have been recorded to be tolerant to sesame gall midge (*Asphondylia sesame*) that causes flower and capsule damage to sesame thus reducing yield (Ogwal *et al.* 2003). Sesim 1 and Sesim 2 are released cultivars in Uganda for commercial production and were officially released because of their high yield performance.

5.4.3 Analysis of Variance across locations

The results of analysis of variance (ANOVA) across locations and seasons are presented in Table 5.6.

Table 5.6: Combined analysis of variance of yield data of 16 sesame genotypes tested in 3 locations for 4 seasons.

Source of variation	D.F	S.S	M.S	F-value	p-value	%variation
Season(S)	3	4107465	1369155***	19.12	<0.001	10
Location (L)	2	12899	6449ns	0.09	0.914	0
Season x Location	6	18088342	3014724***	42.09	<0.001	45
Residual	24	1718873	71620	3.21		
Genotype (G)	15	1934120	128941***	5.77	<0.001	4
Season x Genotype (S x G)	45	1339039	29756ns	1.33	0.083	3.3
Location x Genotype (L x G)	30	1717895	57263***	2.56	<0.001	4.3
Season x Location x Genotype (S x L x G)	90	3076274	34181**	1.53	0.003	7.7
Residual	360	8042700	22341			
Total	575	40037608				

Level of significance: ns=non-significant, ** (P<0.01), *** (P< 0.001%)

There was highly significant difference ($P < 0.001$) among the four seasons suggesting high variability among seasons. This could have been due to some biotic and abiotic factors. Seasons contributed 10% of the total sum of squares for variation. Location showed no significant difference suggesting that locations were similar in conditions. Rahmatollah *et al.* 2013 found no significant effect of location in their analysis. Highly significant difference ($P < 0.001$) was recorded for season x location (S x L) suggesting that there was high interaction between the seasons and locations. Season by location contributed highest in the total sum of squares of variation with 45%. Highly significant difference ($P < 0.001$) was recorded for genotypes indicating that there were differences in yield performance among the genotypes with some genotypes giving high yields while others were poor yielding. Genotypes contributed 4% of the total sum of squares due to variation. Rahmatollah *et al.* (2013) recorded significant effect for genotypes and attributed that to changes in genotype characteristics and Velu and Shunmugavalli (2005) also reported highly significant difference among genotypes. Season x genotype (S x G) did not show significant difference suggesting that high yielding genotypes performed better in all seasons and low yielding genotypes performed poorly in all seasons. Location by genotype interaction (L x G) was highly significant ($P < 0.001$) suggesting that genotypes interacted with locations and performed differently in different locations. The season x location x genotype (S x L x G) interaction showed highly significant difference ($P < 0.001$) suggesting that there was high interaction among genotypes during the seasons in the different locations. Velu and Shunmugavalli (2005) reported similar results.

Partitioning of variance components for environment into predictable (locations) and unpredictable (seasons) is very important (Karimizadeh *et al.* 2013) because when GE interaction is due to variation in predictable factors, a plant breeder has the choice of either developing specific genotypes for selected environments or broadly adapted genotypes that can perform well under variable conditions (Dehghani *et al.* 2006). When GE interaction results from unpredictable sources, a plant breeder needs to develop stable genotypes that perform reasonably well under a range of environmental conditions.

5.4.4 AMMI and Principal Component Analysis (PCA).

AMMI analysis of variance for seed yield in kg/ha of 16 sesame genotypes presented in Table 5.7.

Table 5.7: AMMI analysis of variance for seed yield (kg/ha) of 16 sesame genotypes

Source of variation	df	SS	MS	F	F prob	% of variation
Replication	24	1718873	71620	3.21	0.00000***	
Treatments	191	30276034	158513	7.10	0.00000***	
Genotypes	15	1934120	128941	5.77	0.00000***	6.5
Environments	11	22208706	2018973	28.19	0.00000***	73.4
Interactions	165	6133208	37171	1.66	0.00004***	20.3
IPCA 1	25	2067259	82690	3.70	0.00000***	33.7
IPCA 2	23	1149880	49995	2.24	0.00108***	18.7
Residuals	117	2916069	24924	1.12	0.22414ns	
Error	360	8042700	22341			
Total	575	40037608	69631			

Level of significance: ns=non-significant, *** ($P < 0.01\%$)

The results showed that all the sources of variations that included treatments, genotypes, environments, blocks, interactions, IPCA1 and IPCA 2 were highly significant ($P < 0.001$). Environment contributed the highest total variation of sums of squares with 73.4%. This showed that there was high variability in the environment. Interactions had 20.3%. There was less interaction recorded as compared with Laurentin and Montilla (1999) who recorded 94% of the AMMI model sum of squares attributed totally to $G \times E$ interaction. This difference could be due to the different genotypes and environments used in the two trials. Genotypes contributed low variability (6.5%) for the total variation indicating that genotypes did not change their ranking much under different locations and seasons.

The AMMI biplot showing the main and interaction (PC1) effects of both genotypes and environments on sesame grain yield is presented in Figure 5.3.

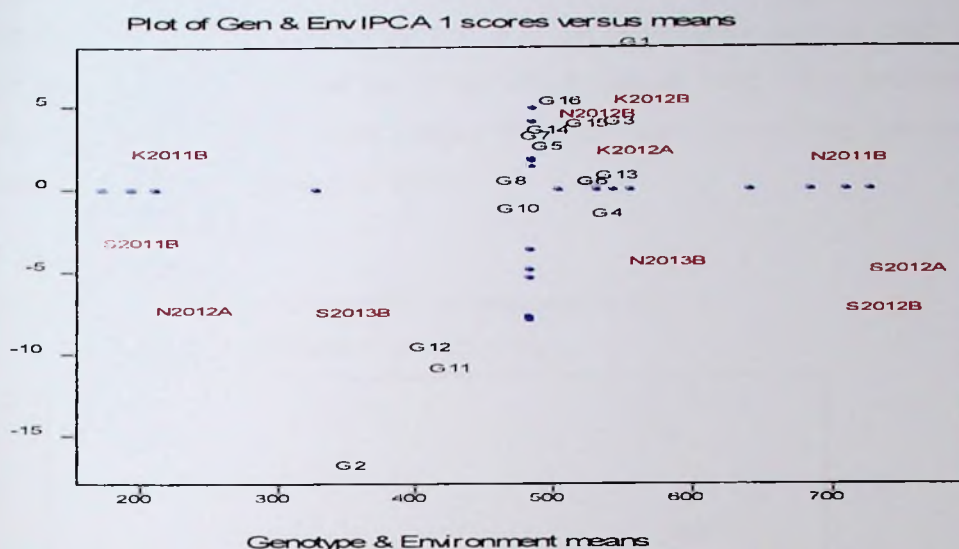


Figure 5.3: AMMI biplot showing the main and interaction (PC1) effects of both genotypes and location by seasons on sesame grain yield.

High yielding genotypes were located on the positive side of the graph, for example: G1 (Ajimo A1-6//7029)-1-1 and G3 (Local 158//6022)-1-2-1. Stable and high yielding genotypes were G4 (Sesim 2//5181)-2-2-1), G6 (Adong 4-4), and G13 (Renner 1-3-1-17) as they were near the origin (Laurentin and Montila, 1999). Stable but poor yielding genotypes were G8 (EM15-3-2) and G10 (Local 158-5) as they are near the origin but on the left side of the vertical line of the genotype and environment means. Poor yielding and unstable genotypes were G2 (Ajimo A1-6//7029)-1-9), G11 (Renner 1-3-14) and G12 (Renner 1-3-1-16) which were far away from the origin and also positioned on the left side of the vertical line for genotype and environment means which indicates areas of low yield. Most of the genotypes evaluated were high yielding as they were above average and on the positive side of the graph except G2, G11 and G12. Stable and high yielding genotypes such as G1, G4, G9, G15 and G16 are good and could be considered as desirable genotypes to be used in the breeding programme. The best performing environments (location x season) were N2011B (Ngetta 2011B), S2012A (Serere 2012A), S2012B (Serere 2012B), K2012A (Kaberamaido 2012A), N2013B (Ngetta 2013B), K2012B (Kaberamaido 2012B) and N2012B (Ngetta 2012B). The rainfall generally was good in all these locations

except that in S2011B the trial was planted rather late as land was opened late due to management problem and partly affected the trial. K2011B performance was low partly due to pest damage caused by webworm and gall midge which reduced yield. Also, genotypes and environments that fall in the same sector interact positively while those falling into opposite sectors interact negatively (Farshadar *et al.* 2012).

5.4.5 GGE Analyses

The results of a pattern of “which-won-where” are presented in Fig 5.4.

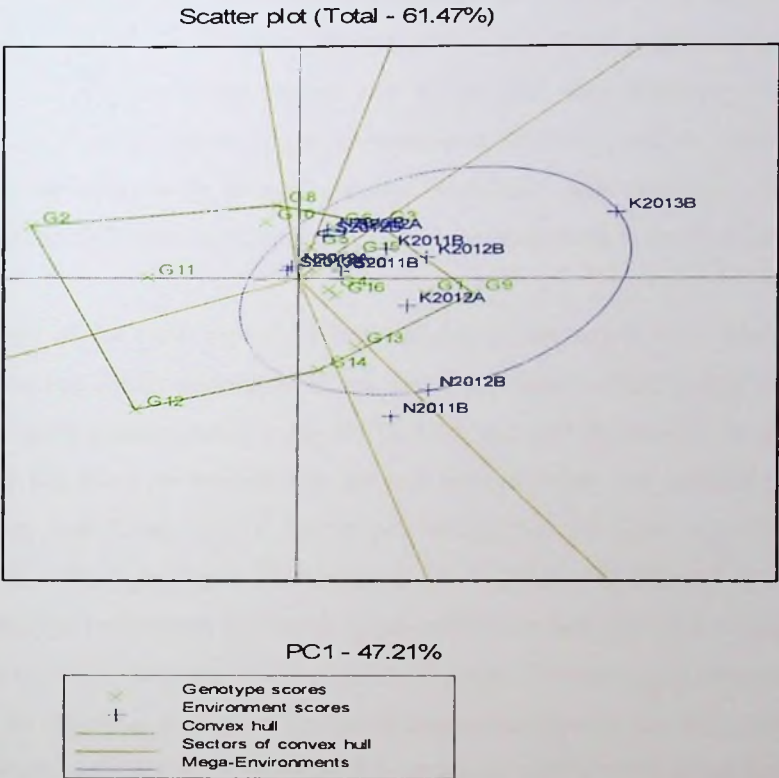


Figure 5.4: Polygon view of GGE biplot analysis showing the mega-environments and their respective high yielding genotypes for ‘which-Won-Where’ pattern.

Based on the three locations and seasons used in this study, only one mega-environment was identified as all environments were located in one sector. Mega environments are test environments with different winning genotypes located at the vertex of the polygon and located in different sectors. Locations and seasons within mega-environment 1 were Kaberamaido

2011B, 2012A, 2012B, 2013B and Ngetta 2011B and 2013B. For this mega-environment, G9 (Local 158-1) was the most positively responsive as it was at the vertex and therefore was the highest yielder in that sector. Although other genotypes were located in other sectors, they did not fall in any environment and therefore those sectors were not considered as mega-environments. Yan *et al.* (2007) reported that if all environment markers fell into a single sector, it would indicate that a single cultivar had the highest yield in all environments and if environment markers fell into different sectors then it would mean that different cultivars won in different sectors. Since a mega-environment is defined as a group of locations that consistently share the best set of genotypes (Yan and Rajcan, 2002), data from multiple years are essential to decide whether or not the target region can be divided into different mega-environments suggesting that in order to create mega-environments, there is need to evaluate genotypes in wider locations far apart with known climatic variability and different soil conditions. The polygon view of the GGE biplot showed that all test environments were divided into 6 sectors.

The polygon view of the GGE biplot explicitly displayed the which-won-where pattern (Yan *et al.* 2001) by showing corner genotypes which are at the vertex of the polygon and are the most responsive genotypes (Karimizadeh *et al.* 2013). They are also the best or the poorest depending on whether they fall in an environment or not and normally they are farthest from the origin of the biplot (Yang and Kang, 2003). Genotypes within the polygon were less responsive to location than the vertex genotypes (Karimizadeh *et al.* 2013). G2 (Ajimo A1-6//7029)-1-9 was the furthest genotype away from the biplot origin and to the left side of the graph which was an indicator of its negative responsiveness to environments. This genotype therefore could not be recommended for growing in any of the environments inspite of the fact that it has desirable attributes for being early maturing because it is seriously affected by Fusarium wilt that makes the genotype to wilt and dry before physiological maturity. Genotypes (G2, G8 and G12) at the vertex were considered to be poor-yielding as they were located far away from the test locations reflecting that they yielded poorly in each season/locations and were therefore not good for these environments. G9 had the highest mean yield (564 kg/ha) among all genotypes across locations and seasons as it was the most positively responsive genotype in the productive environments.

of the environments. The genotype G9 (Local 158-1) was the top yielding genotype, as presented on the front of an average environment towards the pointing arrow of the AEC abscissa. This confirmed the results in Table 5.5. In addition, the biplot indicated that G9 had the highest mean yield but was not one of the stable genotypes as it was not positioned close to the AEC abscissa. At Serere, it was the overall best across the seasons (Table 5.2) with 461 kg/ha while at Ngetta, it was the 5th in ranking although in N2011B, it was the best ranking with 913 kg/ha. Its ranking was reduced in N2012B as it performed poorly in that season compared to most genotypes. In Kaberamaido, it also ranked as the best across seasons with 700 kg/ha (Table 5.4). Across locations and seasons (Table 5.5), it had the highest mean yield with 564 kg/ha. The second highest yielding and most stable genotype was G1. G12 was the most unstable as it had the longest vector. In Table 5.2, at Serere, it ranked as the second last with 338 kg/ha and at Ngetta (Table 5.3), it ranked number seven with 510 kg/ha and at Kaberamaido (Table 5.4), it again ranked the second last. Generally, across locations (Table 5.5), it was the second last among genotypes. Farshadfar *et al.* (2012) explained that genotypic PC1 scores >0 classified the high yielding genotypes while PC1 < 0 identified low yielding genotypes. Unlike PC1, genotypic PC2 scores near zero exhibited stable genotypes whereas large PC2 scores discriminated the unstable ones. S2011B, K2011B, K2012A and K2012B were stable environments while N2011B and N2012B were the most unstable environments. K2013B was the most productive but responsive location and season suggesting that it is high yielding in a particular environment but not stable across environments. K2013B was a discrimination vector as it was the most responsive environment. The smaller the absolute length of projection of a genotype, the more stable it is (Yan, 2002). However, considering both mean yield and stability concepts, plant breeders need to explore genotypes that indicate yield stability as well as high yield across environments (Kang, 2002).

Discriminating vector showing ability of an environment is presented in Figure 5.6.

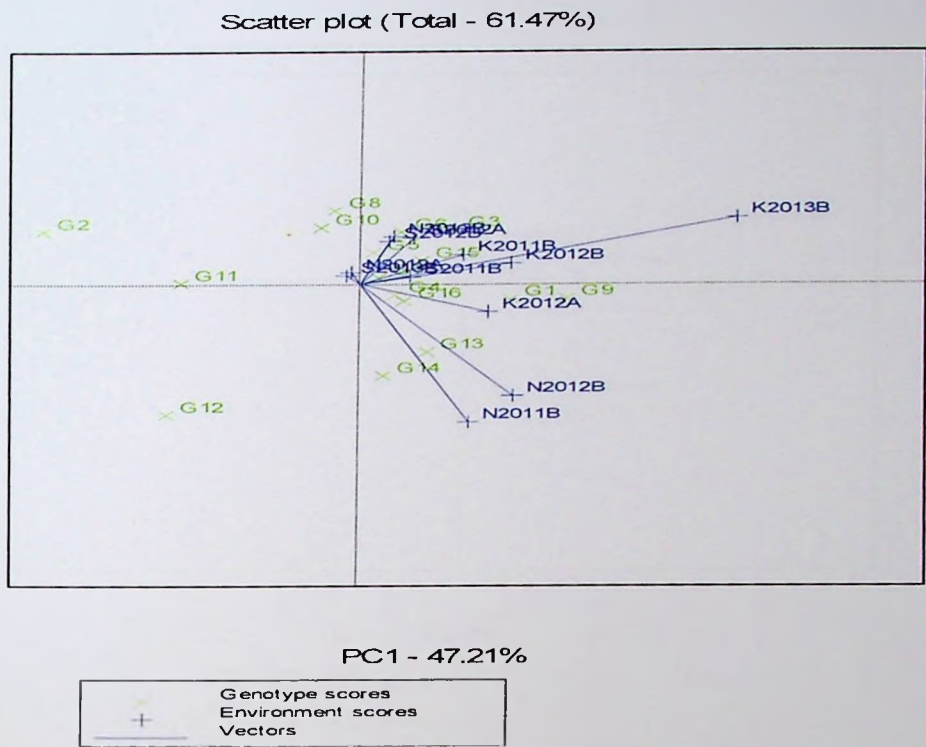


Figure 5.6:The environment vector biplot of 16 genotypes for yield.

K2013B had the longest vector from the origin and therefore was highly discriminating for the genotypes tested and K2013B had the highest mean yield of 638 kg/ha and the lowest coefficient of variation of 24% (Table 5.4). Most of the genotypes recorded very high yields in that location during that season, for example, G9 had the highest yield of 963 kg/ha. The angles between the environments were less than 90^o indicating that most environments were positively correlated to each other. Another interesting observation from the vector view of the biplot was that the length of the environment vectors was approximate to the standard deviation within each environment, which was a measure of their discriminating ability (Yan & Kang. 2003).

The GGE-biplot for comparisons of the genotypes with the ideal genotype is presented in Figure 5.7.

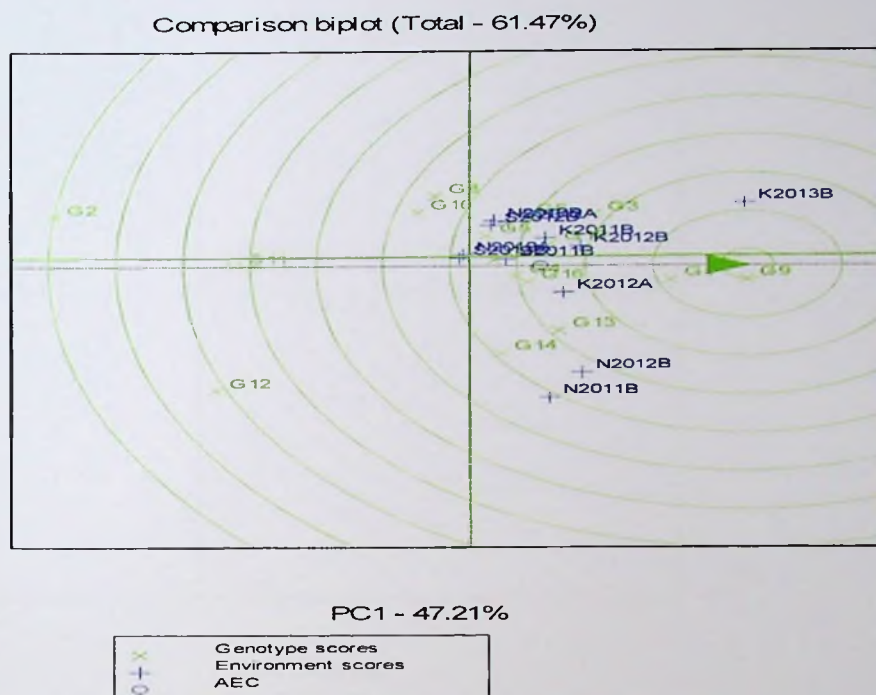


Figure 5.7: GGE-biplot for comparison of the genotypes with the ideal genotype .

The center of the concentric circles is where an ideal genotype (high mean yield and the most stable one) should be located (Yan, 2002) as the projection of the ideal genotype on the average tester coordinate (ATC) horizontal axis is equal to the longest vector of all genotypes and its projection on the ATC vertical axis is obviously zero (it is absolutely stable). Genotype G9 followed by genotype G1 which were closest to the concentric center indicated ideal genotypes which were the most yielding and stable genotypes. This compared favourably with results obtained in Table 5.5 where G9 was the best yielder across locations with 564 kg/ha followed by G1 with 523 kg/ha. Genotypes G3, G4, G5, G6, G7, G13, G14, G15, G16 were considered desirable genotypes because they are within the circles that surround the circle of the virtual ideal genotype. Genotypes G2, G12 and G11 were the genotypes furthest from the ideal genotype and so considered as the poorest yielding genotypes. Similar results were reported in Table 5.5.

Comparison of environments to an ideal environment is presented in Figure 5.8.

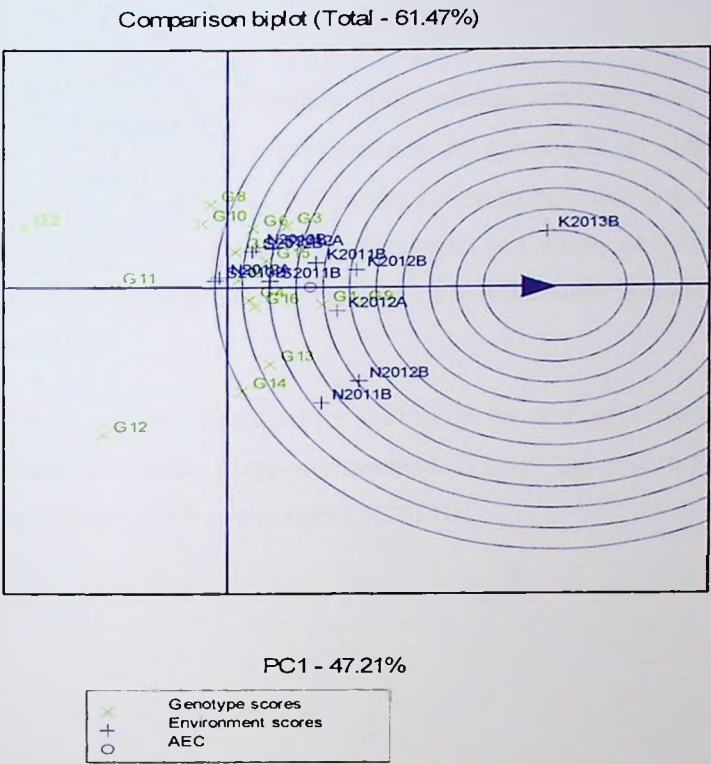


Figure 5.8: GGE-biplot for comparison of environments with the ideal environment.

In this experiment, the ideal environment was also represented by the central circle with an arrow passing through it and it showed that K2013B (Kabera maïdo 2013B) was the closest to ideal environment and therefore the most desirable of all the seasons and most effective season for selecting superior genotypes. Similar results were reported in Table 5.4 where K2013 had the highest mean yield across the seasons in Kabera maïdo. It also indicated that two genotypes (G9 and G1) had yields of over 900 kg/ha. K2011B, K2012A, K2012B and N2012B were also desirable environments as they were located in the middle parts of the concentric rings towards the ideal environment. Ngetta 2012A, S2012A, N2013B and S2013B were the worst seasons (environments) as they were the furthest from ideal environment which was in the innermost concentric rings. Yan and Rajcan (2002) defined an ideal test environment as having small PC2

scores (more representative of the overall environment) and large PC 1 scores (power to discriminate).

5.5 Conclusions

Different genotypes performed differently in the seasons and locations. The GGE model aided in the determination of the relative performance of genotypes at different environments and identification of genotypes suitable for groups of environments. The results also identified the mega-environments as well as the winning genotypes in the mega-environments. The AMMI and GGE biplot gave visual interpretations that particularly allowed visualization of any crossover in the GE interaction.

One mega-environment was identified for sesame in the trial sites with G9 (Local 158-1) being the highest yielder in the main mega-environment. G2 was the best in N2012A and G13 was the only genotype suitable for the environment N2011B.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General Discussion

6.1.2. Genetic diversity

From the 10 tested chloroplast (cp) primer pairs, only *ccmp2* revealed allelic variation. With this primer pair (*ccmp2*) that showed variability in the number of alleles among accessions, 3 different haplotypes (Haplotype A, B and C) were detected. Fourteen out of the seventeen accessions belonged to haplotype B. Only 4 accessions had haplotype A. Within these 4 accessions, two of them, EM15-1-15 and Ajimo AI-6//7029-1 showed haplotype A only while ICEASE 00019 showed haplotype A and C in a ratio of 7:1. It was also noticed that, haplotype C was only found in this accession (ICEASE 00019). Local 158//6022-1-2-1 showed haplotypes A and B in a ratio of 5:3. Occurrence of different haplotypes in an accession indicated that there was foreign pollination in such accessions. This was also an indication that natural outcrossing took place in sesame crop. Locus *ccmp2* therefore, had a discriminatory power in identifying more than one haplotype in accession.

For EST-SSR analysis, starting with 3,328 ESTs from NCBI dbEST, 36 primer pairs were generated for subsequent testing with 24 primer pairs revealing good PCR-products, but only 8 loci showed genetic variation in the test set of 17 accessions. CL93Contig 1 had the highest major allele frequency (0.97) and Bu670267 had the least (0.42). Bu670267 had the highest heterozygosity (H_e) of 0.73 and also had the highest PIC (0.69) among the EST-SSR markers selected suggesting the superiority of this locus in revealing the genetic diversity in these accessions. Major allele frequency was high ranging from 0.42 in BU670267 to 0.97 in CL93Contig1. Although BU670267 had the lowest major allele frequency, it was recorded in eight genotypes compared to loci which were recorded only in two or three genotypes. It also had seven alleles while other loci had only two alleles. The expected heterozygosity and polymorphic information content (PIC) for this locus were the highest with 0.73 and 0.69 respectively. It was observed that where major alleles were high, the expected heterozygosities

(H_e), observed heterozygosities (H_o) and PIC were low. High presence of major alleles therefore did not provide high information and genetic diversity in the accessions.

By using a dendrogram based on Nei's genetic distance to understand the genetic relationships between the 17 tested accessions, the results revealed that the 17 germplasm lines fell into four different clusters, where three accessions from ICRISAT formed a clade on their own. Another observation was that accessions which shared phenotypic similar traits, especially hairiness formed together in a cluster.

For the genetic analysis made on the 115 sesame land races separated from the breeding lines, nine loci were scored. These were sampled from 13 districts in Uganda. Heterozygosity and polymorphism were calculated for each locus and for each accession separately. The mean H_o values of each locus were very low (0 – 0.184), mean H_e values ranged from very low to rather high (0.004 – 0.683). The low H_e values in this study could be partly explained by the fact that the microsatellites were developed from ESTs, which are generally less variable than those from non-coding regions of the genome. Accession-wise, the calculated Shannon diversity index (I) ranged from 0 to 0.624 (grand mean = 0.298), indicating various depths of genetic diversity within the accessions. The mean H_e values (or gene diversity, D) varied from 0 to 0.396 (grand mean = 0.084), and the H_o values ranged from 0 to 0.299 (grand mean = 0.194) suggesting low diversity within the accessions.

The overall estimate of gene flow (Nm) among populations of Ugandan sesame accessions was rather high with 4.476, which might be due to a high out-crossing rate (Pathirana, 1994;), and/or because of anthropogenic effects such as extensive seed trade.

An AMOVA analysis was used to group accessions into the respective districts. The majority of the variance was occurring among the individuals accounting for 76 % of the total variation, and only 5 % of the variation was attributed to differences among the districts. Similar results where differences among geographical regions were represented by only 5 % of the total variation in sesame accessions were described by Laurentin and Karlovsky (2006) and Woldeesenbet *et al.* (2015).

Principal coordinate analysis showing genotypic diversity of landraces from different districts in Uganda where the sesame accessions were collected revealed that the accessions were mixed without any pattern from one region to another indicating that seeds were transported from one region to another with no border. The land races, therefore, could not be grouped according to regions but according to the phenotypes such as plant height, maturity, capsule length and size and branching type.

The genetic diversity based on nuclear marker for breeding lines showed a total of 9 loci scored and at each locus, the mean H_o values were very low (0.003 – 0.197) and mean H_e values were in the range of 0.013 – 0.380. The low H_e values in this study could be partly explained by the fact that the microsatellites were developed from ESTs, which were generally less variable than those from non-coding regions of the genome. Shannon diversity index (I) ranged from 0 to 0.655 (grand mean = 0.313) and the mean H_e values (or gene diversity, D) varied from 0 to 0.373 (grand mean = 0.200), and the H_o values ranged from 0 to 0.382 (grand mean = 0.097). These results were in line with previous studies, where a gene diversity (H_e , D) between 0 and 0.440 was reported in African sesame lines (Gebremichael and Parzies, 2011; Nyongesa *et al.*, 2013; Sehr *et al.*, 2016). In the dataset of 83 accessions, the following 4 accessions showed no gene diversity at all (H_e , I , H_o = 0): 036 (China), 067 (USA), 079 (Kenya) and 118 (Uganda).

The degree of variation within a specific country of origin, measured by the Shannon's diversity index (I), ranged from a low value of 0.237 up to 0.936. The number of alleles ranged from 1.444 – 4.778, and the highest allelic richness was seen in the lines from Kenya and the USA. Lines from Korea, Tanzania, and Zimbabwe showed very few numbers of alleles. The overall estimate of gene flow (N_m) between the countries of origin was 1.387, which was less than what was seen in Ugandan landraces where a gene flow of 4.476 was detected among different districts (Sehr *et al.*, 2016). This indicated some gene flow among accessions from different countries of origin since the value still exceeded 1. This amount of gene flow, which was naturally due to either seed dispersal and/or via cross-pollination, could be also explained by anthropogenic effects such as extensive seed trading and deliberate introductions by plant breeders.

An AMOVA to evaluate the diversity components within and among the accessions, which had been grouped into the respective countries of origin revealed that the majority of the variance occurring among the individuals accounted for 69 % of the total variation, and 11 % of the variation was attributed to differences among the countries of origin. This was an indication that there was high variability among individuals within the same group compared to variability among different accessions.

The principal coordinate analysis of the agro-morphological traits and genetic diversity clustered in their respective country of origin and revealed a pattern associated to the main geographic origins. The accessions from the USA overlapped with those from China while the accessions from Uganda overlapped with those from Ethiopia indicating similarity in their origin.

The results of genotypic analysis of 200 sesame lines for F-statistics and estimates of N_m over all populations for each locus showed that *ccmp2* had high F_{is} and F_{it} at unity and F_{st} at 0.868 but had the lowest N_m . The mean values of F_{is} , F_{it} , F_{st} and N_m were 0.514, 0.790, 0.596 and 0.031 respectively. All the loci had high F_{it} and F_{st} above 0.5 except CL569Contig1, CL93Contig1, GBssr_sa_108 and GBssr_sa_184. N_m was generally low for all the loci. The highest was CL93Contig1 with 0.304 and the lowest was *ccmp2* with 0.038. The fixation index (F_{st}) values were generally high (0.451 to 0.868) indicating that high levels of genetic differentiation were present in the sesame populations sampled. " F_{st} " is the most appropriate index of gene-frequency differentiation if the genetic diversity is low (Nagylaki, 1998). The value ranged from 0-1 where zero implied complete panmixis and a value of one meant that the populations did not share any genetic diversity and that all the variation arising from them can be explained by their population structure (Holsinger *et al.*, 2009).

Various outcrossing rates were described in sesame, ranging from less than 1 % to over 50 % (Andrade *et al.*, 2014). Such a high degree of variation necessitated studies under local conditions in order to clarify the outcrossing rate in Uganda. From the six different plants investigated, only one plant had seeds that showed no introgression. The other five plants showed varying degrees of introgression by foreign pollination. The level ranged from 3.1 % up to 62.5 % of foreign alleles with a mean value of 26.5 %.

6.1.2 Gene Action

Two experiments were conducted to analyse combining ability and gene effects for yield and yield components using diallel and line x tester mating designs. While another experiment using generation mean analysis was designed to study the gene action and their interactions among crosses. Hybrid crop production appears to promise a yield breakthrough in sesame like in other self-pollinated crops such as rice, barley, wheat and could make sesame more competitive and could lead to wider cultivation and greater returns

A diallel mating design of an 8 x 8 crossing was used and the eight parents and their 28F1 crosses were evaluated in a RCBD. The genetic variance of combining ability was separated into general (GCA) and specific (SCA) combining ability variance components and Baker's ratio, coefficients of genetic determination (CGD) were determined for narrow and broad sense heritabilities. High GCA effects were recorded for days to flowering, height of first capsule and number of branches, branches per plant and capsule length. Desirable SCA effects were recorded for Sesim2//5181x Renner 1-3-1-1 for reduced height of first branch, Sesim1 x Sesim2 for number of branches per plant, capsules on branches and yield per plant. High Baker's ratio was recorded for most of the traits except for yield per plant (0.233). CGD_{bs} was high for most of the traits except for days to maturity (0.064), plant height, capsules on main stem and capsule width. The results suggested that both additive and non-additive gene actions played a major role in the traits. Sesim 1 had the highest number of desirable traits (8) scored for GCA effects and could be considered as the best parent for crossing to produce desirable progeny. Sesim 1 x Sesim 2, Sesim 1 x Ajimo A1-5 and Sesim2//5181 x SPS1438-1-6-4 recorded positive significant SCA effects for yield per plant and, therefore, could be used for hybrid seed production.

Line x tester mating design showed that mean squares across locations had high significant difference for all the traits recorded indicating that locations had effect on the traits. Variances for the males were higher than the variances for the females for all the traits except capsule width suggesting greater variability among the males than the females indicating that the introduced accessions that were used as males had greater variability. Combined general combining ability (GCA) across locations showed that GCA for the lines and testers were significantly positive for a number of traits. Specific combining ability (SCA) was only significantly positive for days to

flowering, number of capsules on branches, number of capsules on main stem, total number of capsules and capsule length. Baker's ratio was high for most of the characters and medium for plant height, number of capsules on branches, number of capsules on main stem and capsule length. Coefficients of genetic determination for narrow sense heritability (CGD_{ns}) were high for days to flowering, height of first branch and height of first capsule. Among the lines, Sesim 2, Ajimo A1-5, EM15-1-5, Ajimo A1-6//7029, Local 158//6022, (Y-1//Local 158)-1-2-1 and among testers 4036-1-10-2 and SPS1438-1-6-4 were desirable for different traits. The results suggested that the improvement of these yield contributing traits may not be feasible with simple breeding methods indicating the need for reciprocal recurrent selection followed by pedigree selection (Joshi 1979) or diallel selective mating design suggested by Jenson (1970).

The additive, dominance and epistatic types of gene interaction in each cross for the different traits such as length of capsule zone, number of branches, number of capsules on branches and plant height were found to be different from each other. The simultaneous occurrence of the additive and dominance gene action makes it necessary to use recurrent selection to harness the different kinds of gene effects. The additive effects and gene interaction dominance x dominance (I) or other digenic complementary gene interaction can be exploited effectively by use of biparental mating.

6.1.3. Genotype by Environment Interaction

Field experiments were conducted with 16 genotypes for four seasons (2011-2013) at three locations of Serere (NaSARRI), Kaberamaido and Ngetta. ANOVA, AMMI and GGE were applied to analyse the data. AMMI analysis of variance for seed yield (kg/ha) showed that IPCA1 and IPCA 2 were highly significant ($P < 0.001$). The combined analysis of variance indicated that season, season x location, genotype and location x genotype had significant ($P < 0.001$) variation. The GGE biplot suggested the existence of only one sesame mega-environment with genotype G9 (Local 158-1) winning in that mega-environment followed by G1 (Ajimo A1-6//7029)-1-1. The mega-environment had environments K2011B, K2012A, K2012B, N2012B and K2013B. The vertex genotypes which were the most responsive in their respective environments were (Ajimo A1-6//7029)-1-9, (Local 158//6022)-1-2-1, EM15-3-2, Local 158-1, Renner 1-3-1-16 and Renner 1-3-1-17-1. Genotypes (Ajimo A1-6//7029)-1-9 and Renner 1-3-1-

16 performed poorly in poor environments. Genotypes were categorized into stable and high yielding, stable but poor yielding, unstable but good yielding and unstable and poor yielding. Environment K2013B was the most discriminating environment. Genotype Local 158-1 was the best genotype and Kaberamaido was the nearest to ideal environment. G9 (Local 158-1) was officially released in 2013 as Sesim 3 variety for commercial production because of its high yield, stability, tolerance to pests and high oil content.

6.2 Conclusions

There was no pattern of distinction among the local landraces as far as districts and regions were concerned in Uganda but some distinction could be observed among the land races, breeding lines and introductions. The tight clustering of the local landraces indicated that they were possibly mainly duplicates with different names.

Genetic diversity analysis based on molecular data (SSRs) and agro-morphological traits revealed a high degree of genetic polymorphism in Ugandan sesame landraces and a high degree of gene flow ($N_m = 4.476$) among the districts but no obvious relationship between geographical origin and grouping based on genetic and morphological similarities was observed.

The combining ability and gene action results suggested that both additive and non-additive gene actions played a role in the traits

AMMI and GGE biplots were effective in visualizing the response patterns of genotypes and environments. Variability in yield and stability were recorded among genotypes but the locations seemed to be similar as only one mega environment was identified.

6.3. Recommendations.

Breeders should look for higher number of alleles which is an indication of the discriminating power of the loci in identifying the accessions that have the potentials to be used in the breeding program at the National Semi-Arid Resources Research Institute (NaSARRI) through crossing different accessions and using loci with high mean allele numbers. Varietal purity should be maintained in sesame germplams by selfing to avoid out-crossing.

Landraces should be crossed with introduced accessions mainly from USA and China since they cluster differently indicating that they have different genetic background with different phenotypic traits not available in the landraces.

Sesim 1 with the highest number of desirable traits scored for GCA effects should be a main parent for crossing in order to produce desirable progeny. Sesim 1 x Sesim 2, Sesim 1 x Ajimo A1-5 and Sesim 2//5181 x SPS1438-1-6-4 which recorded positive SCA effects for yield per plant could be considered for hybrid seed production.

Stability of performance should be considered as an important aspect of yield trials and both stability and yield must be considered simultaneously so as to make selection of genotypes more precise and reliable. The AMMI and GGE biplots are statistical methods that should be adopted by plant breeders since they can easily visualize and separate genotypes which are stable and high yielding.

It is important to evaluate genotypes in environments with different agroecological conditions so that the mega-environments are identified with different genotypes for different seasons and locations.

Due to outcrossing by insects especially bees, seed production fields for pure seed should be beyond 200 meters from other sesame varieties.

In order to maintain high yields, farmers should sought for high yielding varieties from Seed Companies and not just buy mixed seeds from their local markets.

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APPENDICES

Appendix 1: List of the sesame landraces used in the evaluation

No	Accession code	Name of the accession	Sub-county	County	District	Region
1	Code 28	Arut Kidetok	Kasilo	Serere	Soroti	Teso
2	Code 29	Kidetok SPSS	Kidetok	Serere	Soroti	Teso
3	Code 88	No name	Asuret	Soroti	Soroti	Teso
4	Code 89	Acare	-	Soroti	Soroti	Teso
5	Code 90	missing	-	-	-	Teso
6	Code 91	No name	Kalaki	Kaberaimaido	Kaberaimaido	Teso
7	Code 92	No name	Otuboi	Kaberaimaido	Kaberaimaido	Teso
8	Code 114	Local Sesim 2	Asuret	Soroti	Soroti	Teso
9	Code 124	Arut	Kaberaimaido	Kaberaimaido	Kaberaimaido	Teso
10	Code 125	Arut	Kaberaimaido	Kaberaimaido	Kaberaimaido	Teso
11	Code 139	Arut	Kaberaimaido	Kaberaimaido	Kaberaimaido	Teso
12	Code 166	Otara	Kaberaimaido	Kaberaimaido	Kaberaimaido	Teso
13	Code 26	Oyam local-3	Loro	Oyam south	Oyam	Lango
14	Code 27	Oyam local-4	Loro	Oyam south	Oyam	Lango
15	Code 30	Otara Awelo-2	Not known	Awelo	Amolatar	Lango
16	Code 31	Otara Awelo	Not known	Awelo	Amolatar	Lango
17	Code 32	Ajimo Amuca	-	Erute North	Lira	Lango

18	Code 93	No name	Bata	Dokolo	Dokolo	Lango
19	Code 94	No name	Bata	Dokolo	Dokolo	Lango
20	Code 95	No name	Bata	Dokolo	Dokolo	Lango
21	Code 96	No name	Abako	Moroto	Lira/Alehtong	Lango
22	Code 97	No name	Abako	Moroto	Lira/Alehtong	Lango
23	Code 98	Local Sesim 2	Abako	Moroto	Lira/Alehtong	Lango
24	Code 99	No name	Abako	Moroto	Lira/Alehtong	Lango
25	Code 100	No name	Aloi	Moroto	Lira/Alehtong	Lango
26	Code 101	No name	Aloi	Moroto	Lira/Alehtong	Lango
27	Code 102	Ajimo	Aloi	Moroto	Lira/Alehtong	Lango
28	Code 103	No name	Aloi	Moroto	Lira/Alehtong	Lango
29	Code 104	Ajimo	Adekokwok	Erute North	Lira	Lango
30	Code 105	No name	Adekokwok	Erute North	Lira	Lango
31	Code 106	No name	Adekokwok	Erute South	Lira	Lango
32	Code 107	No name	Adekokwok	Erute South	Lira	Lango
33	Code 108	No name	Ogur	Erute North	Lira	Lango
34	Code 109	No name	Ogur	Erute North	Lira	Lango
35	Code 110	No name	Ogur	Erute North	Lira	Lango
36	Code 111	No name	Agwata	Dokolo	Dokolo	Lango
37	Code 112	Ajimo	Adekokwok	Erute North	Lira	Lango
38	Code 162	Oturutata	Ngai	Oyam North	Oyam	Lango
39	Code 163	No name	Ngai	Oyam North	Oyam	Lango
40	Code 164	Oturutata	Ngai	Oyam North	Oyam	Lango
41	Code 165	Acare	Ngai	Oyam North	Oyam	Lango
42	Code 167	Ajimo Areng	Iceme	Oyam North	Oyam	Lango

43	Code 168	No name	Aboke	Oyam North	Oyam	Lango
44	Code 169	No name	Aboke	Oyam North	Oyam	Lango
45	Code 194	Otara Areng	Aber	Oyam South	Oyam	Lango
46	Code 195	Otara Arut	Loro	Oyam South	Oyam	Lango
47	Code 196	Otara Arut	Loro	Oyam South	Oyam	Lango
48	Code 198	Otara	Bala	Kole	Kole/Apac	Lango
49	Code 199	Acara	Kangai	Dokolo	Dokolo	Lango
50	Code 200	Acara	Bata	Dokolo	Dokolo	Lango
51	Code 113	No name	Puranga	Aruu	Pader	Acholi
52	Code 115	Oturutata	Puranga	Aruu	Pader	Acholi
53	Code 116	Bur obia	Puranga	Aruu	Pader	Acholi
54	Code 117	Bur obia	Awere	Aruu	Pader	Acholi
55	Code 118	Local Sesim 2	Awere	Aruu	Pader	Acholi
56	Code 119	Bur obia	Awere	Aruu	Pader	Acholi
57	Code 120	Anyena	Awere	Aruu	Pader	Acholi
58	Code 121	Oturutata	Awere	Aruu	Pader	Acholi
59	Code 122	Oturutata	Awere	Aruu	Pader	Acholi
60	Code 123	Oturutata	Awere	Aruu	Pader	Acholi
61	Code 126	Oturutata	Kilak	Aruu	Pader	Acholi
62	Code 127	Bur obia	Kilak	Aruu	Pader	Acholi
63	Code 128	Bur obia	Lapul	Aruu	Pader	Acholi
64	Code 129	Bur obia	Lapul	Aruu	Pader	Acholi
65	Code 130	No name	Lapul	Aruu	Pader	Acholi
66	Code 131	No name	Lapul	Aruu	Pader	Acholi
67	Code 132	No name	Lapul	Aruu	Pader	Acholi

68	Code 133	Ajimo	Pajule	Aruu	Pader	Acholi
69	Code 134	Oturutata	Pajule	Aruu	Pader	Acholi
70	Code 135	No name	Paicho	Aswa	Gulu	Acholi
71	Code 136	Oturutata	Paicho	Aswa	Gulu	Acholi
72	Code 137	Oturutata	Paicho	Aswa	Gulu	Acholi
73	Code 138	Oturutata	Labonga Amida	Chua	Kitgum	Acholi
74	Code 140	Oturutata	Labonga Amida	Chua	Kitgum	Acholi
75	Code 141	Renge	Labonga Amida	Chua	Kitgum	Acholi
76	Code 142	No name	Labonga Amida	Chua	Kitgum	Acholi
77	Code 143	No name	Labonga Amida	Chua	Kitgum	Acholi
78	Code 144	No name	Labonga Amida	Chua	Kitgum	Acholi
79	Code 145	No name	Labonga Amida	Chua	Kitgum	Acholi
80	Code 146	No name	Labonga Amida	Chua	Kitgum	Acholi
81	Code 147	No name	Labonga Amida	Chua	Kitgum	Acholi
82	Code 148	No name	Bungatira	Aswa	Gulu	Acholi
83	Code 149	Oturutata	Lagwiny	Aswa	Gulu	Acholi
84	Code 150	Latyen Ayweri	Lagwiny	Aswa	Gulu	Acholi
85	Code 151	Ajimo Latino	Lagwiny	Aswa	Gulu	Acholi
86	Code 152	Arut	Bungatira	Aswa	Gulu	Acholi
87	Code 153	Arut	Bungatira	Aswa	Gulu	Acholi
88	Code 154	No name	Bungatira	Aswa	Gulu	Acholi
89	Code 155	No name	Bungatira	Aswa	Gulu	Acholi
90	Code 156	No name	Bungatira	Aswa	Gulu	Acholi
91	Code 157	Latupana	Bungatira	Aswa	Gulu	Acholi

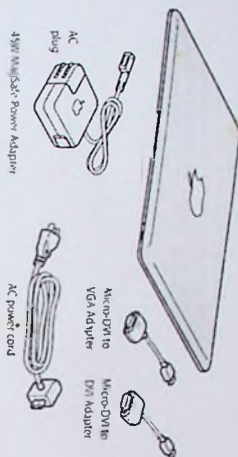
92	Code 158	Ajimo otara	Bungatira	Aswa	Gulu	Acholi
93	Code 159	No name	Lakwana	Omoro	Gulu	Acholi
94	Code 160	missing	-			Acholi
95	Code 161	Oturutata	Lalogi	Omoro	Gulu	Acholi
96	Code 197	No name	Labonga Amida	Chua	Kitgum	Acholi
97	Code 170	No name	Rhino Camp	Madi Okolo	Arua	West Nile
98	Code 171	No name	Rhino Camp	Madi Okolo	Arua	West Nile
99	Code 172	No name	Rhino Camp	Madi Okolo	Arua	West Nile
100	Code 173	No name	Rhino Camp	Madi Okolo	Arua	West Nile
101	Code 174	Odroini	Rhino Camp	Madi Okolo	Arua	West Nile
102	Code 175	No name	Rhino Camp	Madi Okolo	Arua	West Nile
103	Code 176	No name	Ogoko	Madi Okolo	Arua	West Nile
104	Code 177	No name	Ogoko	Madi Okolo	Arua	West Nile
105	Code 178	No name	Ogoko	Madi Okolo	Arua	West Nile
106	Code 179	Arut green	Ogoko	Madi Okolo	Arua	West Nile
107	Code 180	No name	Ogoko	Madi Okolo	Arua	West Nile
108	Code 181	No name	Bileffe	Terego	Arua	West Nile
109	Code 182	No name	Bileffe	Terego	Arua	West Nile
110	Code 183	Aringgaru	Bileffe	Terego	Arua	West Nile
111	Code 184	Lande ogal	Kucwiny	Padyere	Nebbi	West Nile
112	Code 185	Lande Matar	Kucwiny	Padyere	Nebbi	West Nile
113	Code 186	No name	Got Lande	Padyere	Nebbi	West Nile
114	Code 187	No name	Panyango	Jonam	Nebbi	West Nile
115	Code 188	Ogali	Panyango	Jonam	Nebbi	West Nile
116	Code 189	Missing	Panyango	Jonam	Nebbi	West Nile
117	Code 190	No name	Panyango	Jonam	Nebbi	West Nile
118	Code 191	Local Sesim 2	Pakwach Town council	Jonam	Nebbi	West Nile
119	Code 192	No name	Pakwach Town council	Jonam	Nebbi	West Nile
120	Code 193	No name	Panyango	Pakwach	Nebbi	West Nile
121	Code 33	Kigumba local		Kigumba	Masindi	Bunyoro

Appendix 2: List of breeding lines (introductions, crosses, earlier assembled lines in use at NaSARRI

Serial No	Accession code	Name of the accession	Country of origin	Remark
1	Code 1	Sesim 1	Uganda	Released variety/Breeding line
2	Code 2	Sesim 2	Uganda	Released variety/Breeding line
3	Code 8	EM15-3-2	Uganda	Breeding line
4	Code 9	Adong 4-4	Uganda	Breeding line
5	Code 10	AD-1-1-1	Uganda	Breeding line
6	Code 11	U1-7	Uganda	Breeding line
7	Code 12	U1-7-1	Uganda	Breeding line
8	Code 13	EM15-1-5	Uganda	Breeding line
9	Code 14	Ajimo A1-5	Uganda	Breeding line
10	Code 15	Ajimo A1-6	Uganda	Breeding line
11	Code 16	Sesim 2//5181	Uganda	Breeding line
12	Code 17	Ajimo A1-6//7029	Uganda	Breeding line
13	Code 18	Local 158//7029	Uganda	Breeding line
14	Code 19	Local 158//6022	Uganda	Breeding line
15	Code 20	(U1-7 x Eva)-1-1	Uganda	Breeding line
16	Code 21	(Y-1 x Local 158)	Uganda	Breeding line
17	Code 22	(Y-1 x Local 158)	Uganda	Breeding line
18	Code 23	(Y-1 x Local 158)	Uganda	Breeding line
19	Code 24	(Y-1 x Local 158)	Uganda	Breeding line
20	Code 72	Ajimo A1-6//7029	Uganda	Breeding line
21	Code 3	Local 158-1	Ethiopia	Released variety/Breeding line
22	Code 4	Local 158-2	Ethiopia	Breeding line
23	Code 5	Local 158-3	Ethiopia	Breeding line
24	Code 6	Local 158-4	Ethiopia	Breeding line
25	Code 7	Local 158-5	Ethiopia	Breeding line
26	Code 34	Early Russian	Korea	Introduction
27	Code 35	NAL 79.309.16.1	Tanzania	Introduction
28	Code 36	4036-1-1-2-1	China	Introduction
29	Code 37	4036-1-1-2-5	China	Introduction
30	Code 38	4036-1-1-2-6	China	Introduction
31	Code 39	4036-1-2-2-1	China	Introduction
32	Code 40	4036-1-2-2-2	China	Introduction
33	Code 41	4036-1-2-2-3	China	Introduction
34	Code 42	4036-1-2-2-4	China	Introduction
35	Code 43	4036-1-2-3	China	Introduction
36	Code 44	4036-1-2-4	China	Introduction
37	Code 45	4036-1-10-2-1	China	Introduction
38	Code 46	1438-1-6-1	China	Introduction
39	Code 47	1438-1-6-1-1	China	Introduction

40	Code 48	1438-1-6-2	China	Introduction
41	Code 49	1438-1-6-3	China	Introduction
42	Code 50	1438-1-6-10-2	China	Introduction
43	Code 51	1438-1-6-18-1	China	Introduction
44	Code 52	1438-1-10-1-1	China	Introduction
45	Code 53	1438-1-10-2-1	China	Introduction
46	Code 54	1438-1-10-3	China	Introduction
47	Code 55	1438-1-1—3	China	Introduction
48	Code 56	Renner-1-1-1-6	USA	Introduction
49	Code 57	Renner 1-3-1-4	USA	Introduction
50	Code 58	Renner 1-3-1-1-2??	USA	Introduction
51	Code 59	Renner 1-3-1-1	USA	Introduction
52	Code 60	Renner 1-3-1-??	USA	Introduction
53	Code 61	Renner 1-3-1-1	USA	Introduction
54	Code 62	Renner 1-3-1	USA	Introduction
55	Code 63	Renner 1-3-1-1	USA	Introduction
56	Code 64	UCR3-1-2-1	USA	Introduction
57	Code 65	UCR3-1-2-2	USA	Introduction
58	Code 66	UCR3-1-2-3	USA	Introduction
59	Code 67	UCR3-1-4	USA	Introduction
60	Code 68	UCR3-1-5	USA	Introduction
61	Code 69	UCR3-1-6	USA	Introduction
62	Code 70	UCR3-1-7	USA	Introduction
63	Code 71	UCR3-1-8	USA	Introduction
64	Code 73	ICEASE 00001	ICRISAT/Kenya	Introduction
65	Code 74	ICEASE 00002	ICRISAT/Kenya	Introduction
66	Code 75	ICEASE 00003	ICRISAT/ Kenya	Introduction
67	Code 76	ICEASE 00004	ICRISAT/ Kenya	Introduction
68	Code 77	ICEASE 00005	ICRISAT/ Kenya	Introduction
69	Code 78	ICEASE 00006	ICRISAT/ Kenya	Introduction
70	Code 79	ICEASE 00007	ICRISAT/ Kenya	Introduction
71	Code 80	ICEASE 00008	ICRISAT/ Kenya	Introduction
72	Code 81	ICEASE 00015	ICRISAT/ Kenya	Introduction
73	Code 82	ICEASE 00018	ICRISAT/ Kenya	Introduction
74	Code 83	ICEASE 00019	ICRISAT/ Kenya	Introduction
75	Code 84	ICEASE 00020	ICRISAT/ Kenya	Introduction
76	Code 85	ICEASE 00022	ICRISAT/ Kenya	Introduction
77	Code 86	ICEASE 00096	ICRISAT/ Kenya	Introduction
78	Code 87	Otis	Zimbabwe	Introduction
79	Code 25	Oyam hairy	Unknown	Introduction

What's in the Box



Important: Remove the protective film covering the 45W MagSafe Power Adapter before setting up your MacBook Air.

About Optical Discs

Although your MacBook Air doesn't have an optical disc drive, it does include DVD discs with important software. You can easily access this software, as well as install applications and access data from other optical discs, using the optical disc drive on another Mac or Windows computer. You can also use the optional MacBook Air SuperDrive, an external optical disc drive.

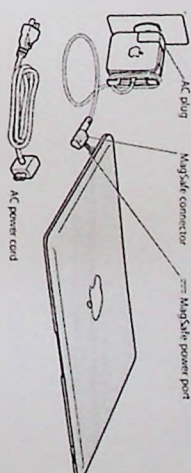
Setting Up Your MacBook Air

Your MacBook Air is designed so that you can set it up quickly and start using it right away. The following pages take you through the setup process, including these tasks:

- Plugging in the 45W MagSafe Power Adapter
- Turning on your MacBook Air and using the trackpad
- Using Setup Assistant to access a network and configure a user account and other settings
- Setting up the Mac OS X desktop and preferences

Step 1: Plug in the 45W MagSafe Power Adapter to provide power to the MacBook Air and charge the battery.

Insert the AC plug of your power adapter into a power outlet and the MagSafe connector into the MagSafe power port, located on the back left side of your MacBook Air. As the MagSafe connector gets close to the port, you'll feel a magnetic pull drawing it in.

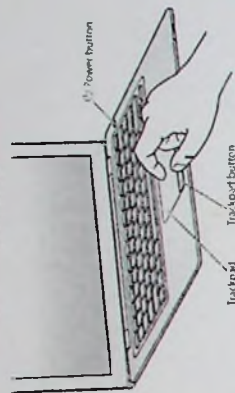


To extend the reach of your power adapter, replace the AC plug with the AC power cord. First pull the AC plug up to remove it from the adapter, and then attach the included AC power cord to the adapter, making sure it is seated firmly. Plug the other end into a power outlet.

When disconnecting the power adapter from an outlet or from the computer, pull the plug, not the cord.

When you first connect the power adapter to your MacBook Air, an indicator light on the MagSafe connector starts to glow. An amber light indicates that the battery is charging. A green light indicates that the battery is fully charged. If you don't see a light, make sure the connector is seated properly and the power adapter is plugged into a power outlet.

Step 2: Press the power button briefly to turn on your MacBook Air. You will hear a tone when you turn on the computer.



It takes the computer a few moments to start up. After it starts up, Setup Assistant opens automatically. If your computer doesn't turn on, see "If your MacBook Air doesn't turn on or start up" on page 42.