Sudan University of Science and Technology
College of Agricultural Studies

Molecular Characterization and Genotypic Variability for Yield and Yield Components in Seventeen Sesame (*Sesamum indicum* L.) Genotypes

التوصيف الجزيئي والتباين الوراثي للانتاجيه ومكوناتها في سبعة عشر سلاله وراثيه لمحصول السمسم

A Dissertation Submitted to the Sudan University of Science and Technology in Partial Fulfillment for the Degree of B.Sc. in Agriculture (Honors)

By:

Hajir Adil Osman Fgir

Supervised by:

Dr: Atif Elsadig Idris

Department of Agronomy

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الآية

قال تعالى:

ٱللَّهُ نَزَّلَ أَحۡسَنَ ٱلۡحَدِيثِ كِتَٰبٗا مُّتَشَٰبِهٗا مَّثَانِيَ تَقۡشَعِرُّ مِنۡهُ جُلُودُ
ٱلَّذِينَ يَخۡشَوۡنَ رَبَّهُمۡ ثُمَّ تَلِينُ جُلُودُهُمۡ وَقُلُوبُهُمۡ إِلَىٰ ذِكۡرِ ٱللَّهِۚ
ذَٰلِكَ هُدَى ٱللَّهِ يَهۡدِي بِهِۦ مَن يَشَآءُۚ وَمَن يُضۡلِلِ ٱللَّهُ فَمَا لَهُۥ
مِنۡ هَادٖ ٢٣

سورة الزمر (23)

DEDICATION

I dedicate this work to my dear father who

did not spare the day with anything.

To my meaning of love, smile to the secret of my success

My lovely mother.

To my brothers and sisters.

To my friends

Hajir

ACKNOLEDGEMENT

First praise to ALLAH for giving me patience and strength to complete This work successfully. I would like to express my sincere and deepest gratitude to my supervisor Dr. Atif Elsadig Idris for his encouragement, understanding and excellent support during my B.Sc. study. I would like to express my thankfulness to Dr. Atif Ibrahim Abu Ali for his kindness and help. Finally, I gratefully thank my family for their excellent support, patience and encouragement. Also thanks to my teachers in Sudan University of Science and Technology, College of Agricultural Studies Department of Agronomy for their advice and guidance of this work.

Hajir

CONTENTS

ABSTRACT

This experiment was carried out in the experiment field of the College of Agricultural Studies, Sudan University of Science and Technology, in Shambat, during the period from the 13thJuly2014 toNeom2014. The goal of this research is to estimate of variability, heritability between different characters in seventeen sesame *(Sesamum indicum* L.) genotypes and used SRAP technology in identifying sesame genotypes. Randomized complete block design (RCBD) with three replications was used in this study. Different yield characters among the seventeen genotypes were measured. The results showed that there were highly significant differences at ($p \leq 0.01$) were scored for weight of 1000 seed, and significant differences at ($p \leq 0.05$) were scored for number of capsules/plant and number of capsules in main stem, and non-significant differences was obtained for seed yield (g) and seed yield (t/ha). The highest values of genotypic and phenotypic variances were scored for number of capsules/plant respectively. Higher value of heritability was recorded for weight of 1000 seed, whereas the lowest value was scored for number of capsules in main stem. The genotype High Land scored the highest yield (0.2506t/ha).

الخلاصة

نفذت التجربة فى الحقل التجريبى بكلية الدراسات الزراعية بجامعة السودان للعلوم و التكنولوجيا, شمبات فى الفترة من13يوليو2014الى نوفمبر2014 بغرض تقدير التباين ودرجة التوريث لسبعة عشر طزر وراثية من اصناف السمسم وصممت التجربة بالقطاعات العشوائية الكاملة بثلاثة مكرارات., تم قياس عدد من صفات الانتاجية,وكذلك تم استخدام التوصيف الجزيئى لهذه الاصناف بواسطة تقنية ال SRAP Technology.اظهرت النتائج وجود فرقات معنوية بين الطرز الوراثية فى وزن الالف بذره, وعدد الكبسولات في النبات,وعدد الكبسولات في الساق الرئيسي,بينما لاتوجد فرقات معنوية فى انتاجية البذور بالجرام انتاجيتها بالطن للهكتار.وان اكبر قيمة التباين الوراثى والمظهري سجلت لعدد الكبسولات فى النبات, كما اثبت الدراسة ان اكبر قيمة لمعامل التوريث سجلت فى وزن الالف بذره بيمنا اقل قيمة لمعامل التوريث سجلت لعدد الكبسولات في الساق الرئيسي , اظهرت الدراسة ان الصنف هايلاند احرزاعلى انتاجية بالطن /هكتار.

CHAPTER ONE

INTRODUCTION

Sesame (*Sesamum indicum* L.) is one of the most ancient and important oil seed crops (Mabberley, et al. 1997). The diploid chromosome number of sesame is (2n=26) and it is usually self-pollinated (Pathirana, 1994). Sesame belongs to the Pedaliaceae family. Which it contains 16 genera and 60 species. Sesame is typically an annual species. There are lots of varieties of (Sesamum indicum L.) according to the size, form and colour of flowers, seed size, colour and composition. Another variation is that some varieties are highly branched where as others are unbranched (Peter, 2004). It probably originated in Africa where both the genus and its family exhibit their greatest diversity (Khidir,2007). Most of sesame produced for the extraction of seed oil but considerable proportion eaten as food in various forms such as sesame seed butter(Gandi,2009).

Sesame is identified as the main rotational crop in rain-fed cropping systems of the Sudan. the crop grown mainly in rain-fed conditions. Based on average cultivated area, in Sudan during 2005-2008, sesame ranks the third after sorghum (7.8 million hectares) and pearl millet (2.3 million hectares). Sesame oil has medicinal and pharmaceutical value and is being used in many health care products .Sesame seed contains about 35-63% oil and17-28% protein with natural antioxidant a such as sesamin,sesamolin and sesamol which has been used as active ingredients in antiseptics , bactericides, viricides, disinfectants, moth replants, anti-tubercular agents (Bedigian et al .,1985).In addition, sesame seed is rich in tryptophan and methionine (Johnson et al.,1979) and it is a good source of essential and beneficial micronutrients required for healthy growth (Obiajunwa et al.,2005).what's more, the high quality sesame oil cake is used as protein source in animal feeding. According to FAO

statistics (2008), the global cultivated area of sesame was about 7.4 million hectares producing about 3.4 million metric tons which makes it the fifth most important oil seed crop on an area basis worldwide.FAO(2005-2008) reported that the Sudan grows about 20.12% (1.48 million hectare) of the world cultivated area , and contributes about 9.24% (0.32 million metric tons) of the total production . moreover, the Sudan acknowledged as the second world exporter of sesame in 2006 and third one in 2007, but unfortunately it is not listed with the top sesame oil exporter in the same period . Sesame breeders in the Sudan deduced that local landraces reached its yield plateau since marginal increase in yield is achieved. However, it is estimated that for most crop species, less than 5% of the biodiversity known to exit is being utilized in agriculture, particularly in the case of self-pollinated crops (Tanksley and McCouch,1997),as well, human activities and extreme changing in environmental factors might affect the genetic structure of sesame populations in different ecological zone. The improve varieties have been selected from segregated materials of crosses between local landraces and introduced cultivars(Khidir, 2007). Ahmed et al. (2003). Average yields in the Sudan is extremely low (0.21 t/ha) compared to the(0.47 t/ha) (world average) and (1.15 t/ha) in China. Also, production level are extremely variable . the low productivity and instability of total production could be attributed to a wide range of biotic and a biotic factors, in which the lack of suitable high yielding varieties is at the fore front (Khidir, 2007). Successful breeding program depends on the complete knowledge and understanding of the genetic diversity within and among genetic resources of the available germplasm of sesame . this enable plant breeders to choose parental sources for hybrid

production or for generation of diverse population s for selection. (Ibrahim, 2010).

The objectives of this study were:

1. Study genetic variability in seventeen genotypes of sesame for some yield characters .
2. Estimate heritability and genetic advance for yield

3.Characterize the seventeen sesame genotypes by molecular methods (SRAP) .

4.Identify the DNA of seventeen sesame genotypes by molecular characterization.

CHAPTER TWO

LITERTURE REVIEW

2.1Background:

Sesame (*Sesamum indicum* L.) is an oil crop and the historical evidence suggests the emergence of sesame in Ethiopia and then it was transferred to India , China, and became a common food in the south of Europe, northern eastern Africa and South Asia. Its cultivation spread in many countries of the world between latitudes 40 degrees north and 40 South's. Sesame was introduced to the United States of America in the seventeenth century, and flourished grown very rapidly in many countries of Latin America, such as Mexico, Guatemala, Nicaragua and Venezuela (Khidir, 2007).

Is one of the most ancient crops .It is grown in tropical and subtropical areas on 6.5 million hectares worldwide, producing more than three million tons of seeds India, Sudan, Myanmar and China are the most important sesame producers with 68 % of the world production. Sesame seed, which is highly nutritive (50% oil and 25% protein), is traditionally used for direct consumption and as a source of oil of excellent quality due to the presence of natural antioxidants such as Sesamin and sesamolin the Potentially beneficial effects of sesame on human health have recently renewed the interest for this ancient crop.

2.2Uses of sesame:

Sesame seed, paste and oil are utilized in a very wide range of edible products. Crude sesame oil pressed from the seed can be used directly as cooking oil, while refined oil is used as a salad oil or wherever an edible oil of good keeping quality is needed. Sesame seeds are used in various food preparations, raw or roasted. Throughout the Arab world the seed is crushed into a tasty paste called ‘Tahinia’. The mixture of seeds with sugar and flour is called ‘halwa’. Toasted seeds are consumed in soups or mixed with caramelized sugar can be shaped into candies. Seeds are often sprinkled on cakes, rolls and cookies before baking. Oil is used in the manufacture of margarine and compound cooking fats. As salad oil, it is often combined with other edible oils. In India the oil is used as a component of vegetable ghee and for anointing hair and skin. It is further used as a carrier for medicines and perfumes and as a synergist for pyrethrum-based insecticides. Poor grades sesame seeds are used in the production of soaps, paints, lubricants and lamp-oil. Sesame cake is an excellent livestock feed and a raw material for several foodstuffs. Young leaves are used as a soup vegetable in sub-Saharan Africa. In southern Africa the leaves are smoked as a substitute for tobacco. The ash of the stem is a substitute for salt, and is viewed as a good source of minerals. Dry stalks are used as fuel and as construction material, for building shelters. Various plant parts are used in native medicine in Africa and Asia for a variety of ailments. Mucilaginous leaves or leaf sap are used to treat fever, as a remedy for cough and sore eyes and to kill head lice; the sap is taken to facilitate childbirth, to treat dysentery and gonorrhea and is used in dressings after circumcision. In eastern and southern Africa the leaves play a role in the treatment of snakebites and malaria, in India and

China in the treatment of cancers. Ash from burned stems is used as a medicinal salt. The oil is used to treat cough and earache, and as an emmenagogue and abortifacient. Sesame seeds are valued for their laxative effect.

2.3Botanical characteristics:

Sesame grown to include 16 quarterly Alsemsahalty genus and about 60 species, genus and sesame includes 36 species, mainly species with 2n=26 chromosomes.

2.3.1Root system

Root and guided deep ranges between 120 cm in light soil and 80 cm in heavy soil.

2.3.2Shoot system

2.3.2.1Stem

Sesame herbal plant ranges in length between $^{1}/_{2}$ m -3 meters and leg ribbed, branched, smooth or covered Asairat short. Depends number of branches on the product and plant density and environmental conditions and the most important rate rains. Arise branches of the axilla of Securities may be given other branches preliminary Avraa secondary in part the upper part of the leg.

2.3.2.2Leaves

There is a significant difference in the size and shape of leaves among the items in the plant per Valaorac Lower broad oval shaped, lobed and opposite sometimes either the upper leaves is narrow and Rmohah shape and reciprocal . Advantage of the plant sesame growth is limited, any vegetative growth continues into the growth phase fruiting. In phases

Alnamwalakhirh holds plant flowers in the upper part of the leg and in the main Alafia side.

2.3.2.3Flower

The flower of sesame is hermaphrodite pentagonal Walter Cape campaniform shape and carrying a single paper on the linkages, and there are on each side of the flower Rahiqih gland may be given flowers sometimes ranges so that the number of flowering 1-3 Fe linkages by category . Central flowers open before flowering side, and given the fruits of the biggest ones and Addiimar juggle the product and the efficiency of photosynthesis.

Flower white, pink, purple color and there is a relationship between the color of flowers and the color of the seed producing varieties of color seeds mysterious dark color. The formation of a couple of stamens in some varieties there is a fifth sterile stamen.

Amid overhead consists of Krbeltan Mmelthmtin holds the pen end of May Masim two branches.

Flowers blooming in the early morning and begin to fall in the afternoon and fall all the flowers in the sunset, begins firing pollen with flower opening and lose their ability to fertilization after 24 hours have the stigma ready Csab 24 hours before the open flower and loses its fertility after 48 hours of the flower opening, and vaccination self now there ratio of aqueous vaccination by bees ranging between 3.1% -6.7 %.

2.3.2.4 Fruit (capsule):

The top rectangular gully by Muesli or covered Balsairat by category by four or more compartments length about 2-5 cm split when dry Mmayudy dispersion of seeds .The fruit contains the seed of 50-100 or more.

2.4 Chemical composition:

The sesame seeds contain 50% oil, on average, ranging between 40 % -60 % by cultivar, Containing 20% -30% proteins and calcium (1%) and phosphorus (0.7 %) and vitamin (E).

The proportion of carbohydrates ranging from (14% -22%) different cultivars.

2.5 Phenotypic variability in sesame:

Sesame wide variability is one of the causes that let Langham and Wiemers (2002) avowed that sesame is a plant breeder dream. So sesame cultivars vary considerably among themselves. Single character often varies on the same plant or the same branch. This is for shape of leaves, position of flower, number of fruits per axils. Cultivars of sesame differ considerably from each other in their branching habit, flower color, in the size, shape also it in arrangement of capsules and in color and size of seeds (Bedigian and Harlan, 1983).

Ong' injo and Ayiecho(2009) studied thirty four genotypes of sesame results revealed existence of significant differences among the genotypes for number of days to flowering, plant height, first capsule height, number of branches per plant, number of capsules per plant,1000 seeds weight, seed yield per plant, seed yield per hectare and oil content.

Kuol(2004)examined seventeen sesame genotypes at two locations and reported significant differences in days to 50% flowering, days to

maturity, plant height, ranchers per plant, number of capsules per plant, number of seeds per capsule,1000- seed weight, yield plant and seed yield(kg/ha). Baydar(2005) tested variability in eight sesame genotypes in two seasons, the results showed significant differences for plant height(cm) ,first capsule height(cm), number of capsules per plant, number of seed per capsule, 1000-seed weight(g) and seed yield(Kg/ha)

2.6 Heritability:

Heritability values are helpful in predicting the expected progress to be achieved through the process of selection. Genetic coefficient of variation along with heritability estimate provides a reliable estimate of the amount of genetic advance to be expected through phenotypic selection (Wright, 1921).

Heritability ranged from 49.14% for number of capsules plant^{-1} to 90.17% for number of branches plant^{-1}. According to Singh (2001), heritability values greater than 80% are very high, values from 60 to79% are moderately high, values from 40 to 59% are medium and values less than 40% are low, he reported the characters, like number of branches plant^{-1} and seed yield plant^{-} had very high heritability.

This indicates that selection will be the best step for selecting sesame genotypes having these traits with very high heritability. This is because there would be a close correspondence between the accessions and the phenotype due to the relative small contribution of the environment to the total variability. Similar results were reported by Sumathi and Muralidharan (2009, 2010) for days to maturity. All the remaining characters revealed moderate to high heritability.

High heritability combined with low genetic advance was observed for number of seeds per capsule(Thangavel et al.,2000).In days to 50% flowering ,days to maturity, number of branches per plant, and seed yield

per plant, a high heritability and a low genetic advance were observed(Gangdhara Rao, 2005).

2.7 Molecular markers:

Molecular markers are commonly used in genetic diversity analysis, genetic map construction, gene mapping and cloning, and marker assisted selection in plant breeding. Based on detection procedure ,most molecular marker technologies can be classified into hybridization-based or PCR-system .

2.8Sequence Related Amplified Polymorphism (SRAP):

Sequence related amplified polymorphism is a simple PCR-based technique. SRAP provides amplification of open reading frames. In this technique, only two primers are used. They are 17-21 bp length and their sequences are random. Primers contain AT-, GC- rich cores to amplify intragenic fragments. SRAP primers consist of two parts. The first part is called the core sequence (13-14 bp) and it has no specific constitution. Core sequence is followed by three selective nucleotides at 3' end. Core sequences must be different in forward and reverse primers
(Agarwal, et al. 2008, Li and Quiros 2001). Sequencing studies showed that SRAP polymorphisms are derived from two events. The first are fragment size changes because of insertions and deletions which result in co dominant markers. The second are nucleotide changes which result in dominant markers (Agarwal, et al. 2008). SRAP markers are used for multiple aims in different crops such as map construction, gene tagging and genetic diversity studies. The application of this technique is simple and cheap. The SRAP marker system gives reliable results. However, it gives multiple band results and therefore can be complex to analyze. There is a wide range of applications of SRAP technology such as genetic

map construction, genetic diversity analysis, gene tagging and cloning. Since SRAP detects multiple loci in one reaction, it is feasible to construct ultra dense genetic maps with over10,000 SRAP molecular markers. SRAP has advantages over other molecular detection techniques in gene tagging and cloning and allows screening thousands of loci shortly to pinpoint the genetic position underlying the trait of interest. Sequencing SRAP products enhances the application s of SRAP technology. In well characterized genomes, SRAP sequences are used to identify the chromosomal region of mapped genes while in species without a known whole genome sequence, sequences of SRAP markers on a genetic map allow arranging sequence contigs and assembly of a whole genome sequence.

In sesame, Zhang et al ., (2014b) performed genetic diversity analysis using SRAP and SSR markers. They analyzed 404 land races from a sesame collection in China. Using 11 SRAP and 3SSR markers, they produced 175 fragments, of which 126 were polymorphic with an average polymorphism rate of 72%. They calculated several parameters such as Jaccard s genetic similarity coefficients, Nei s gene diversity and Shannon s information index and constructed a dendrogram with all the 404 landraces. According to the dendrogram, landraces from different agro-ecological zones did not cluster together, suggesting that geographical locations did not represent the greater genetic variation among the sesame landraces. They concluded that SRAP markers would be useful to study sesame genetic diversity and understand the relationship of those indigenous landraces, which would guide the collection, protection and utilization of sesame landraces in breeding purposes.

CHAPTER THREE

MATERIALS AND METHODS

3.1 The Experimental Site (Shambat) description:

The study was conducted the experimental farm, College of Agricultural Studies, Sudan University of Science and Technology (Shambat) during the period from 13 July 2014 to November 2014. Shambat is located at longitude32º-35ºE, latitude15º-40ºN, and altitude 280m above sea level. The climate of the locality is semi arid, with low relative humidity; the temperature varies between45º C maximum and 21ºc in summer and 15º c in winter (Adam 2002).

The soil of the experiment site is described as loam clay. It is characterized by a deep cracking moderately alkaline with low permeability low nitrogen content. P h of 7.5-8 and a high exchange able sodium percentage (ESP) in subsoil (Abdelhafiz2001).

3.2 Plant materials:

The plant material treatments used the study is consisted of 17 genotypes of sesame (*Sesamum indicum* L.) table (3-1) .They were obtained from Agricultural Research Corporation (ARC).

Table (3.1) list of 17 genotypes of sesame (*Sesamum indicum L)* used in the study:

Genotype	Origin
Bash	Local variety collected by ARC
High Landh	Ethiopianvarietyintroducedby ARC
Carawy	Local variety collected by ARC
DanabAlgamal	Local variety collected by ARC
Soliamani	Local variety collected by ARC
Om Shagara	Local variety collected by ARC
Alsodana	Local variety collected by ARC
Abraway	Local variety collected by ARC
Alradom	Local variety collected by ARC
$5_{(3)}$	Landrace variety collected by ARC
Harba 15	Local variety collected by ARC
J4	Introduced variety by ARC
$13_{(3)}$	Landrace variety collected by ARC
Kenana	Released variety by ARC Variety
Tagarib	Inbred line by ARC
Tailandi	Introduced variety by ARC

ARC: Agricultural Research Corporation.

Abbreviations of sesame genotypes used for Molecular Characterization in this study:

S1	**Om shagara**
S2	**Solaimani**
S3	**Caraway**
S4	**Harba15**
S5	**17**
S6	**$5_{(3)}$**
S7	**J4**
S8	**Kenana**
S9	**Danab Algamal**
S10	**Tailandi**
S11	**$13_{(3)}$**
S12	**Basheen**
S13	**Alradom**
S14	**High Land**
S15	**Tagarib**
S16	**Alsodana**
S17	**Abraway**

3.3Land preparation, Design and layout of the experiment:

The land of experiment was deep ploughed with disc plough followed by disc harrow and then leveled, and divided to ridges inside plot 2×3meters, each plot consists of four ridges70cm apart, the experiment comprised of 51 plots. Seed were sown in holes and the spacing between the holes was 25 cm.

The design of experiment was a Randomized Complete Block Design (RCBD)with three replication. All plots were irrigated at sowing at sowing date of it 13/7/2014 the second on 15/7/2014, then four irrigations was scheduled every 10-12days intervals for the whole experiment. Nitrogen fertilizer was applied to experiment at the rate of (80kg/ha)for each plot separately in two doses , one dose after one month from sowing date, and the second dose after month of adding the first dose.

3.4 Data Collection:

For data collection the following yield characters were measured after the plants at each plot reached physiological maturity.

3.4.1 Number of capsules in branches:

Number of capsules in the branches was counted from five plants selected randomly in each plot and then average number of capsules in the branches per plant was calculated.

3.4.2 Number of capsules in main stem:

Number of capsules in the main stem was counted from five plants

selected randomly in each plot and then average number of capsules in the main stem per plant was calculated.

3.4.3 Weight of 1000 seed (g):

Thousand seed were taken randomly from total seeds of each plot, then weighed and recorded as average weight for 1000 seeds.

3.4.4 Seed yield (t/ha):

The total seed of plants in ten plants in each plot was weight and recorded.

Then yield tan / ha was calculated according to the following formula:

Seed yield tan/ ha= $$\frac{\boldsymbol{sample\ seed\ yield(g)\times 10000(m^2)}}{\boldsymbol{2\times 0.7(m^2)\times 1000}}$$

3.5 Analysis of variance:

The recorded data were subjected to individual analysis of variance for randomized complete block design, to determine the extent of variation among the genotypes. Described by Gomez and Gomez (1984).

3.6Coefficient of variation (C.V):

It was determined for each character studied as the following formula:

C.V= $$\sqrt{\frac{\boldsymbol{mean\ squarea\ of\ error}}{\boldsymbol{Grand\ mean}}}\times 100$$

3.7. Standard error (S.E):

It was calculated as the following formula= $\sqrt{\frac{\boldsymbol{mean\ squarea\ of\ error}}{\boldsymbol{r}}}$

3.8. Comparison between genotypes:

The means were separated using the least significant difference (L.S.D) at 5% level of significant according to the formula=

L.S.D= $\sqrt{\frac{\boldsymbol{error\ mean\ squarea}}{\boldsymbol{r}}}\times \mathbf{t}$

Where: r = number of replications.

3.9. Phenotypic (Ph^{s2}) and genotypic (g^{s2}) variances.

They were estimated from individual analysis of variance (ANOVA) Table as follows:

Phenotypic variance (g^2 ph) =$\boldsymbol{g^{s2}+e^{s2}}$

Genotypic variance (δ^2g) = $\frac{\boldsymbol{m2-m1}}{\boldsymbol{r}}$

where:

r= number of replications. e^{s2}=Error or environmental variance.

M2= mean squares of genotypes M1= mean squares of error

3.10. Genotypic coefficients of variation(GCV):

They were computed according to the formula suggested by Burton and Devan (1953) as follows:

Genotypic coefficients of variation (GCV %) = $\sqrt{\frac{Genotypic\ variance}{Grand\ mean}} \times 100$

3.11. Heritability:

Heritability (h^2) in broad sense was estimated for each character following to procedure of Johanson et .al (1955) as following:

$H^2 = \frac{\delta 2g}{\delta 2ph} \times 100$

Where:

h^2=heritability $\delta^2 g$= genotypic variance

$\delta^2 ph$= Phenotypic variance

3.12 Molecular experiments:

DNA Extraction Protocol (SRAP Extarction Method)

1. Harvest the leaf tissues (2 weeks old) in zip loc bags in a cooler with ice.

2. Warm the Extraction buffer at 65ºC water bath

- 50 mM Tris, pH 8.0.
- 25 mM EDTA.
- 1 M NaCl.
- 1% CTAB

3. Add 5µl of Mercaptoethanol per 5ml warm extraction buffer.

4. Place the leaf tissues (1-5 leaves) in a 15ml centrifuge tube, add the extraction buffer (a total of 5ml) then mix in a blender. Make sure that the buffer is mixed effectively with the pressed tissue sample. Collect the sap extraction

5. After done with leaf samples of one genotype, clean the blender with de-ionized tap water and wipe well with the paper towels.

6. Place the tubes containing extracts in a water bath set at 60ºC for 1 hr. Gently invert the tubes to mix the extract for every 20mins during this incubation time.

7. Remove the tubes from the water bath and let it cool down for 5-10 min (cool to at least 30ºC). Cooling can be hastened by placing the tubes in room temp.

8. Add equal volume (5ml) of freshly prepared Chloroform/isomayle alcohol (24:1).

9. Invert the tubes gently for several times and then let it set for at least 30 min. (or gently mix by inversion for 5-10 min.).

10. Spin the tubes in a table top centrifuge at 6000 rpm for 10 min. (at room temp. or 4ºC).

11. Collect the upper supernatant in a new 15ml tubes using P5000 pipette and 5ml tips (with wide-cut tip).

12. Very gently add 2/3 volume (about 3ml) of cold isopropanol (2-propanol).

13. Slowly tilt and invert the tubes so that DNA will precipitate.

14. Let it to set for at least 30 min. If DNA precipitation is big, no need to wait for 30 min. (OR Overnight is better)

15. Spin for 10 min. at 4000 rpm (14,000 if micro).

16. Discard the supernatant carefully, don't let drain the DNA pellets.

17. Wash the DNA pellets with cold 70% ethyl alcohol (2-3ml) and spin for 1-2 min. at slow speed (2000 rpm). Discard the supernatant.

18. Wash the pellets again with 70% ethyl alcohol, spin for 30 sec. at low speed.

19. Air dry the pellets briefly by inverting the tubes for 5 min.

20. Add 1 ml of TE buffer (10 mM Tris + 1 mM EDTA, pH 8.0), slowly stir the DNA until it dissolve (incubation at 65ºC oven for 5 min. enhance dissolving) or (can be left overnight at 4ºC).

21. Add 5µl of RNase A (10mg/ml). Incubate at 37ºC water bath for 30-60 min. (or leave it overnight at room temp.) (OR @ 37ºC incubator for 1 hr).

22. Add 1/10 volume (200µl) of 8M ammonium acetate & add 2 volumes (2ml) of cold ethanol (95-100%) (you can mix the NH4.Acet+Ethol before use and put in the fridge). Mix by gentle inversion to precipitate the DNA.

23. Let it set for at least 30 min. (OR overnight is better). Spin at 2000 rpm for 8-10 min.

24. Discard the supernatant carefully and air-dry the pellets by inverting the tubes in a clean paper towel.

25. Add 500µl (depending on the size of the pellet) of TE buffer (pH 7.5). Keep it in 4ºC overnight or at 65ºC for 5min.

26. Transfer the DNA to the 1.5ml eppendorf tubes for long term storage.

27. Quantify the DNA on spectrophotometer or fluorometer or using mass ladder.

28. Prepare working stock at a conc. of 10ng/ml of DNA in sterile H_2O

CHAPTER FOUR

RESULTS

4.1 Phenotypic Variability:

The statistical analysis of variance revealed significant differences at ($p \leq 0.01$) for weight of 1000 seed. Significant differences at ($P \leq 0.05$) for number of capsules per plant and non- significant differences for Seed yield per plant (g) and seed yield (t/ha), (Table: 4-2).

4.1.1 Number of capsules per plant:

The highest value for number of capsules per plant (75.267) were exhibited for the genotypes Alradom, while the minimum (17.667) was attended by genotype Bash, the grand mean was (45.093) and the coefficient of variation (35.85) %(Table: 4-2).

4.1.2 Number of capsules in main stem:

The highest value of number of capsules in maim stem (46.88) were exhibited for genotype Bash , while the lowest value (17.53) was attended by genotype Danab Algamal, the grand mean was (30.224) and coefficient of variation (34.01) % (Table 4-2).

4.1.3 Weight of 1000 seed (g):

Table (4.2) shows highest values of 1000 weight (3.8) was obtained the genotypes J4. The lowest value (2.0) was achieved by two genotypes Tagrib, Caraway. The grand mean was (2.91) and the coefficient of was (1.92) %(Table: 4-2).

4.1.4 Seed yield per plant (g):

The highest value of seed yield g (43.86g) was observed on the genotypes High Land. While the lowest values of seed yield (12.43g) were obtained by genotypes DanabAlgamal. The grand mean was (0.1766).While the coefficient of variation was (45.5) % (Table4.2).

4.1.5 Seed yield (t/ha):

The highest value of seed yield t/ha (0.2506t/ha) was observed on the genotypes High Land. While the lowest values of seed yield (0.1766) were obtained by genotypes DanabAlgamal. The grand mean was (0.0721).while the coefficient 0f variation was (45.51) % (Table4-2).

4.2Phenotypic (δ^2ph) and genotypic (δ^2g) variance:

The results showed that phenotypic variances values were higher than the genotypic variances for all yield traits (Table 4-3). The highest value of phenotypic variance (817.24) was scored by the number of capsules per plant. The lowest value (0.0007486) was obtained by seed yield/plant (t/ha). For genotypic variances the value (98.231) were scored by the number of capsules/plant. The lowest value (0.00010266) was obtained by seed yield/plant (t/ha).

Table (4.1). Mean squares from the individual analysis of yield traits it is of seventeenth genotypes of sesame (*Sesamum indicum* L.) grown at Shambat in season2014-2015.

Characters	Mean squares of blocks	Mean squares of genotypes	Mean squares of error
No. of capsules	**355.51**	**555.964***	**261.27**
No. of capsules in main stem	**189.65**	**185.834***	**105.64**
Weight of 1000 seed (g)	**9.35**	**0.733****	**1.97**
Seed yield per plant (g)	**28.42**	**283.44^{Ns}**	**199.08**
Seed yield (t/ha)	**0.00102**	**0.00954^{Ns}**	**0.00646**

**highly significant difference

*significant difference

N_S =not significant difference

Table (4-2) Mean of different traits of seventeen sesame genotypes grown at Shambat 2014-2015.

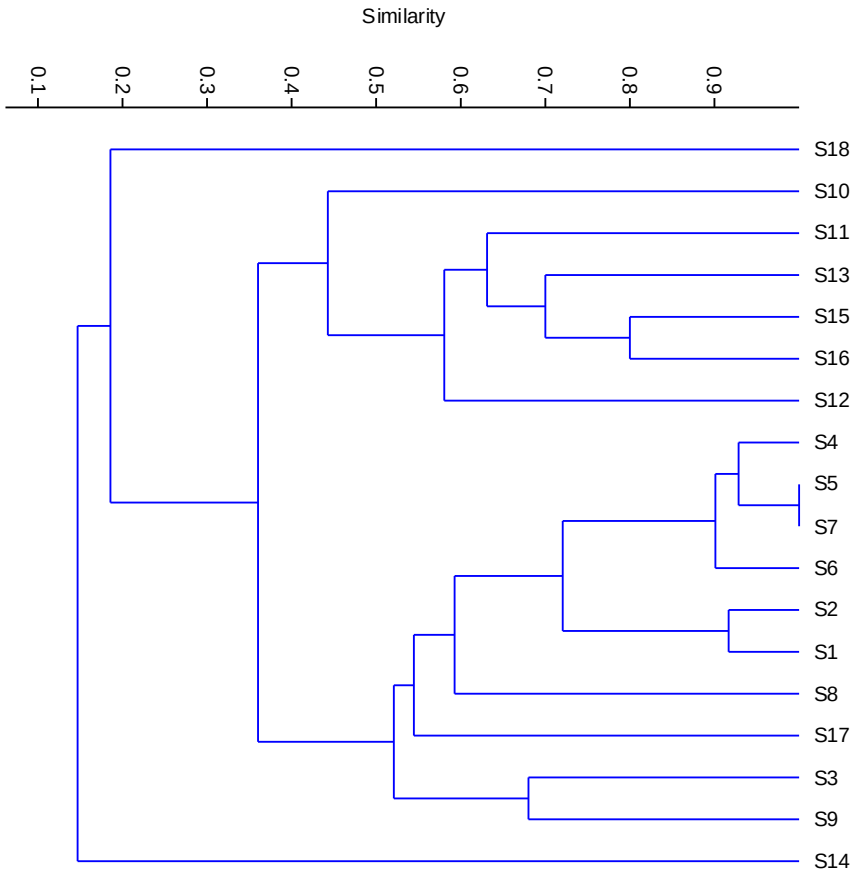
Genotypes	No .of capsules	No .of capsules in main stem	Seeds yield (g)	Seeds yield (t/ha)	Weight of 1000 seed
Abraway	43.800	21.033	38	0.2171	3.2
Om shagara	62.533	31.400	29.63	0.1693	2.8
Alsodana	48.867	21.800	27.6	0.1577	2.6
Danab Algamal	53.800	17.533	12.63	0.0721	2.9
Kenana	34.107	31.533	20.20	0.1154	2.4
Alradom	75.267	42.467	41.33	0.2360	3
$13_{(3)}$	39.933	37.267	24.10	0.1377	3.4
Caraway	60.200	32.800	39.06	0.2232	2
Bash	17.667	46.867	38.46	0.2198	3.2
J4	53.400	28.467	38.40	0.2194	3.8
Harba15	45.667	27.733	42.53	0.2440	3.4
High land	39.500	38.300	43.86	0.2506	3
Tailandi	37.333	29.133	25.53	0.1459	2.5
17	42.600	28.200	39.23	0.2242	3.4
Solaimani	48.300	31.900	19.80	0.1131	3
$5_{(3)}$	32.200	26.867	27.10	0.1549	2.8
Tagarib	31.400	20.500	19.66	0.1024	2
Grand Mean	45.093	30.224	31.01	0.1766	2.92
C.V	35.85	34.01	45.50	45.51	3.83
SE	9.3322	5.9340	11.52	0.656	2.54
LSD	13.198	8.3919	23.467	0.1337	132

Table (4-3) Phenotypic (PCV %) and Genotypic coefficient of variation (GCV %), phenotypic and genotypic variance for the deferent characters studied on seventeen genotypes of sesame evaluated at Shambat in season 2014 - 2015.

Characters	Phenotypic variance	Genotypic variance	Genotypic coefficient of variation (GCV)%	Phenotypic coefficient of variation (PCV)%	Heritability $(h)^2$
No .of capsule	817.235	98.231	147.594	425.715	12.019
No .C. in main stem	291.471	26.732	94.046	310.543	9.171
Seed yield (g)	227.2063	28.119	95.224	270.681	12.375
Seed yield (t/ha)	0.0007486	0.0001026	2.4110	6.5109	13.71
Weight of 1000 seed	4.294	3.892	115.419	121.233	90.63

PRIMERS= OPL18; OPR10

Jaccard’s similarity dendrogram:



Matrix: Minimum similarity= 0.00 (S14/S5; S14/S6; S14/S7) Maximum similarity= 100% (S5/S7)

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18
S1	1.00																	
S2	0.92	1.00																
S3	0.50	0.52	1.00															
S4	0.73	0.79	0.59	1.00														
S5	0.67	0.71	0.55	0.93	1.00													
S6	0.71	0.77	0.50	0.86	0.92	1.00												
S7	0.67	0.71	0.55	0.93	1.00	0.92	1.00											
S8	0.50	0.53	0.56	0.60	0.63	0.67	0.63	1.00										
S9	0.43	0.45	0.68	0.52	0.55	0.57	0.55	0.56	1.00									
S10	0.14	0.14	0.26	0.17	0.18	0.19	0.18	0.24	0.21	1.00								
S11	0.44	0.46	0.61	0.52	0.48	0.44	0.48	0.40	0.45	0.42	1.00							
S12	0.23	0.24	0.46	0.26	0.27	0.28	0.27	0.31	0.41	0.43	0.57	1.00						
S13	0.33	0.35	0.41	0.42	0.38	0.39	0.38	0.36	0.41	0.57	0.63	0.48	1.00					
S14	0.04	0.05	0.14	0.04	0.00	0.00	0.00	0.07	0.10	0.39	0.33	0.28	0.28	1.00				
S15	0.31	0.32	0.54	0.38	0.35	0.36	0.35	0.38	0.54	0.40	0.64	0.62	0.68	0.26	1.00			
S16	0.30	0.31	0.52	0.37	0.33	0.35	0.33	0.37	0.52	0.38	0.62	0.65	0.72	0.25	0.80	1.00		
S17	0.50	0.53	0.43	0.63	0.56	0.60	0.56	0.43	0.50	0.25	0.38	0.33	0.52	0.09	0.55	0.52	1.00	
S18	0.11	0.12	0.26	0.10	0.11	0.11	0.11	0.13	0.26	0.17	0.23	0.33	0.17	0.18	0.30	0.29	0.18	1.00

CHAPTER FIVE

DISCUSSION

5.1Phonotypic variability:

The individual analysis of variance of different traits revealed highly significant differences for some of yield traits and non significant differences among sesame genotypes (*Sesamum indicum* L.), evaluated in this study, high variation among sesame genotypes could be attributed to a wide diversity in plant genotypic materials, hence knowledge of existing genetic variation assumes important and will offer a good scope for improvement of irrigated sesame though traditional selection. These results are similar to the findings reported by many workers (Umar-Shaba, 2012, Ukaan2012 Narayanan, 2010).

5.1.1Number of capsules per plant:

In this study the genotype Alradom attained the highest value of the number of capsules per plant of (131.27) the results were in differ with who attained the rang from(91) were reported by (Ukaan,2012).

5.1.2Number of capsules in main stem:

In this study the genotype Basheen attained the highest value of the number of capsules in main stem of (46.9) the results were in differ with who attained the range from (41.5) were reported by
(Umar-Shaba, 2012).

5.1.6 Weight of 1000 seed (g):

In this study the genotypeJ4 attained the highest value of the Weight of 1000 seed of (3.8) the results were in differ with who attained the rang from (4.02)were reported by (Umar-Shaba, 2012).

5.1.7 Seed yield per plant (g):

In this study the genotype High Land attained the highest value of the Seed yield per plant of (43.86) the results were in differ with who attained the rang from (21.24)were reported by (Umar-Shaba, 2012).

5.1.8 Seed yield (t/ha):

In this study the genotype High Land attained the highest value of Seed yield (t/ha) (0.2506) the results were in differ with who attained the range from (2.879) were reported by (Umar-Shaba, 2012).

5.2 Phenotypic variance (δ^2ph) and genotypic variance (δ^2g):

In this study the phenotypic variance of all traits showed highest value than the genotypic variances. Similar finding were reported by (Narayanan, 2010)

5.3Genotypic coefficient of variation (GCV %):

The genotypic coefficient of variation GCV all traits showed highest value than the genotypic variances. Similar findings were reported by (Narayanan, 2010).

5.4 Heritability:

This indicates that selection will be the best step for selecting sesame genotypes having these traits with very high heritability. This is because there would be a close correspondence between the accessions and the phenotype due to the relative small contribution of the environment to the total variability. Similar results were reported by (Narayanan, 2010) for days to maturity. All the remaining characters revealed moderate to high heritability.

The phylogenetic analysis of this study distinguished that genotype High Land is out of clusters. The first cluster contained five genotypes as sisters which are $13_{(3)}$, Alradom, Tagarib, Alsodana and Basheen. Also Tailandi and Basheen as 2nd sisters, Tagarib and Alsodana as third sisters. In the other cluster there is five sisters, the first one are Harba15, J4,17, $5_{(3)}$, the second sisters are Solaimani and Om shagara, and the third sisters are $5_{(3)}$, Solaimani, Om shagara, Kenana, Abraway, Caraway and Danab Algamal. The 4^{th} sisters are Caraway and Danab Algamal, the 5^{th} one are Tailandi, $13_{(3)}$, Alradom, Abraway, Caraway, and Danab Algamal. Our results are in agreement with reports of ……..

CHAPTER SIX

CONCLUSIONS

Based on the results obtained from this study it could be concluded conclusion that:

1. Variability obtained in this research between sesame genotypes for yield traits, could be of a great value in any sesame breeding program.

2. The results revealed that high value of heritability, this indicates that selection will be the best step for selecting sesame genotypes having these traits with very high heritability.

3. The genotype High Land scored high value in all yield traits. Therefore it could be used as a parental line in any sesame hybridization program or it could select for farmer as sesame genotype characterize with high yield.

4. In this study, the allelic diversity released by the two primers was sufficient enough to distinguish between the genotypes. It concluded that, the high rate of polymorphism between genotypes revealed by SRAP markers indicated that the method is efficient to analyze genetic divergence and can be used to establish consistent heterotic groups between sesame genotypes.

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