Sudan University of Science and Technology
College of Agricultural Studies

Study of Molecular Characterization and Genotypic Variability For some Growth Characters in Sesame *(Sesamum indicum* L.*)*

دراسة التوصيف الجزيئي والتباين الوراثي لبعض صفات النمو في محصول 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:

Ayat M. Mohammed Ahmed Nasser.

Supervised by:

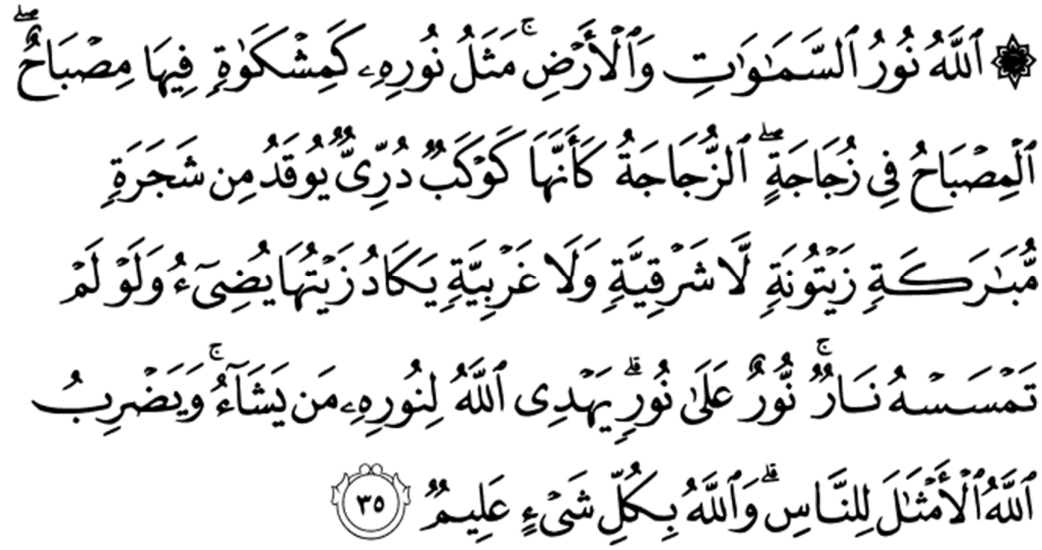
Dr: Atif Elsadig Idris

Department of Agronomy

2015 August

الآية

قال تعالى:



سورة النور

DEDICATION

To My:

Family,

Teachers

And every Friends.

ACKNOWLEDGMENT

First praise and thanks to ALLAH to spire me to work on this topic and giving me strength and patine to complete this work successfully. I would like to express my deepest and sincere gratitude and thanks full to my Great Supervisor: **Dr. Atif. Elsadig Idris** for his very supportness and kind, and a wonderful care in directing and supervising work.

Thanks also to all whom help me in my research.

Finally thanks to my mother for take care and help and hope her health and happiness forever.

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 and correlation between different characters in seventeen sesame *(Sesamum indicum* L.) genotypes and to use SRAP technology in identifying sesame genotypes used in this study. A randomized complete block design (RCBD) with three replications was used in this study. Different growth and yield characters among the seventeen genotypes were measured. The results showed that there were highly significant differences a more genotypes ($p \leq 0.01$) were scored from number of branches, height first capsules, number of capsules, stem diameter, and non- significant differences ($p \leq 0.05$) for, plant height. The highest values of genotypic and phenotypic variances were scored for plant height and height of first capsule respectively. Higher value of heritability was recorded for stem diameter whereas the lowest value was scored for plant height. The genotype Highland scored the highest yield (t/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 الاصناف السمسم وصممت التجربة بالقطاعات العشوائية الكاملة بثلاثة مكررات., تم قياس عدد من صفات النمو ,وكذلك استخدام التوصيف الجزيئى لهذه الاصناف.اظهرت النتائج وجود فروقات معنوية عالية بين الطرز الوراثية فى صفات النمو لكل من عدد الافرع, ارتفاع من اول كسبولة,وسمك الساق , بينما يوجد فروقات معنوية بسيطه فى طول النبات .وان اكبر قيمة للتباين الوراثى والمظهري سجلت بواسطة طول النبات وارتفاع اول كبسوله, كما اثبتت الدراسة ان اكبر قيمة لمعامل التوريث سجلت فى سمك الساق ,و اقل قيمة لمعامل التوريث سجلت فى طول النبات, اظهرت الدراسة ان الصنف هايلاند احزر اعلى انتاجية بالطن/هكتار

CHAPTER ONE

INTRODUCTION

Sesame(*Sesamum indicum* .L) also commonly Known as Beniseed in Nigeria is an important oil seed crop that has been referred to as the Queen of oil seed by the virtue of its high quality oil. It is ancient oil crop that has being cultivated in tropical and subtropical regions of Africa and Latin America. In Nigeria sesame crop is cultivated mainly for its seeds that contain approximately 50% oil and 25% protein. (Burden , D 2005). It is very difficult to judge whether observed variability is highly heritable or not. Moreover, Knowledge of heritability is essential for selection based improvement, as it indicates the extent of trans miscibility of a character into future generations ((Sabesan , et al (2009). Genetic variability and correlation for yield and groin quality characters of rice grown in coastal saline low land of tamilnadu Sesame it probably originated in Africa where both the genus and its family exhibit their greatest diversity (Khidir,2007). Most of sesame produsedis used for the extraction of seed oil but a considerable proportion eatch as food in various forms such as sesame seed butter (gandhi, 2009). According to FAO statistics (2008), the global cultivated area of sesame was about7.4 million hectares, producing about3.4 million metric tons which makes it the fifth most important oil seed crop on an area basis worldwide. Sesame is identified as the main rotational crop in rain-fed cropping systems of the Sudan. The crop grows mainly in rain-fed conditions, in pure stand or intercropped with other crops such as Sorghum, water Mellon, Roselle, cowpea and maize. In Sudan during 2005-2008,sesame ranks the third after sorghum(7.4million hectare) and pearl millet (2.3 million hectare).FAO(2008) reported that the Sudan grows about 20.12%(1.4 million hectare)of the world cultivated area, and contributes

about 9.24%(0.324 million metric tons) of the total production. Moreover, the Sudan acknowledged as the second world exporter of sesame seed in 2006 and third one in 2007, but unfortunately it is not listed with the top sesame oil exporter in the same period.

Sudanese farmers still grows local cultivars along with improved varieties in many areas. Thus, the rate of adoption of new varieties is fairly low. On the other hand; landraces perform well under sub-optimal conditions as they are well adapted and possesses farmer's preferable traits. . Genetic diversity in crop species can be determined by using the agro morphological as well as biochemical and molecular markers (Liu, 1997; Geleta *et al.*, 2007, 2008). Studies on sesame genetic diversity and divergence have been mainly based on agro-morphological traits. Several of these agro-morphological trait based studies have found a high genetic diversity in sesame populations (Bisht et *al.* 1998; Arriel *et al.,* 2007). There is an ample scope for improving the productivity of this important oil seed crop through varietal improvement and hybrid cultivar development. Average yields in the Sudan is extremely low (0.21 t/ha) compared to the (0.47t/ha) (world- average) and 1.15 t/ha in china. Also, production levels are extremely variable. The low productivity and the instability of total production could be attributed to awide rang of biotic and a biotic factors, in which the lack of suitable high yielding varieties is at the fore front. (Khidir, 2007). Any 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 enables plant breeders to choose parental sources for hybrid production or for generation of diverse populations for selection. (Ibrahim , 2010).

Therefore the objectives of this research:

1.Study genetic variability in seventeen phenotypes of sesame for some growth characters .

2. Estimate heritability and genetic advance for growth.

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 .Reaction fragment length polymorphism (RFLP) is the first hybridization-based molecular marker system that was intensively used at the beginning of molecular biology era in life since while hybridization-based marker methods such as microarrays and diversity array technology (DA rT) are used currently to detect single nucleotide polymorphism (SNP). In contrast, many PCR-based molecular marker detection
methods have been developed. For example, amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR) and sequence related amplified polymorphism (SRAP),inter-simple sequence repeat (ISSR), sequence tagged site (STS), and sequence characterized amplification region (SCAR), are commonly used in genomic analysis (Jones et al ., 2009). There are advantages and limitations for all molecular marker detection methods. In particular, RFLP probes can be shared in related species so RFLP is advantageous over other molecular markers in comparative genomics. However, the detection procedure in RFLP is complicated and costly. Additionally, RFLP is not easily automated to analyze thousands of individuals for marker assisted selection. AFLP is a commonly used molecular marker system since it can detect multiple genetic loci in a genome. On the other hand, there are many steps in the AFLP detection procedure, which limits its application in marker assisted selection when thousands of individual DNA samples need to be analyzed in a short time. SSRs often have a high level of polymorphism in plant genomes

and are commonly used in most genomic applications. Since SSR technology only detects sequence repeats, the number of SSRs in a genome is relatively limited compared with numerous SNPs. RAPD is easily per formed in one round of PCR, however, a low level of reproducibility of RAPD amplification limits its wide use in genomic analysis. As next generation sequencing (NGS) technologies dramatically increase capacity and throughput of DNA sequencing, whole genome sequencing of many plant species has been accomplished and most economically important crop species such as rice, maize, soybean, sorghum, potato, tomato and Chinese cabbage have been fully sequenced. Although it is still challenging to use NGS for assembling a whole complex genome such as barley and wheat, there are thousands of SNPs identified in NGS that can be used to develop molecular markers in species with complex genomes. Furthermore, NGS is directly used in SNP discovery and a few dozen genotypes can be sequenced simultaneously to assemble ultra dense genetic maps. Additionally, different strategies are used to produce partial genomes that can be used to directly sequence SNPs using next generation technology.

2.8 SRAP technology:

SRAP technology can be combined with other markers to construct genetic maps. For example, Yu et al., (2007) constructed a high-density genetic map in a cultivated allotetraploid cotton population using SSR, SRAP, AFLP, and target region amplification polymorphism (TRAP). This high density cotton genetic map consists of 697 SSR, 171 TRAP, 129 SRAP, 98 AFLP, and two morphological markers, covering a genetic distance of 4,536.7 c M with the average genetic distance of 4.1 c M per marker. Gulsen et al., (2010) reported a new citrus linkage map using SRAP, RAPD, SSR, ISSR, peroxidase gene polymorphism (POGP), resist 26 Plant Breeding from Laboratories to Fields ant gene analog

(RGA), and a morphological marker, Alternaria brown spot resistance gene. In total, they assembled 385 SRAP, 97 RAPD, 95 SSR, 18 ISSR, 12 POGP, and 2 RGA markers on the citrus genetic map. In the Cucu rbitaceae family, Yeboah et al., (2007) constructed genetic maps in cucumber using SRAP and ISSR markers. They developed pseudo-testcross F1 segregating populations from a cross between two diploid parents and constructed male and female parental genetic maps separately with 164 SSR and 108 SRAP markers. More recently, Zhang et al., (2012) constructed a high density consensus genetic map in an inter-sub specific mapping population in cucumber. The consensus map contained over a thousand molecular markers including 1,152 SSR, 192 SRAP, 21 SCAR and one STS. In another cucurbit species, Levi et al., (2006) constructed an extended genetic map for watermelon using five PCR-based molecular markers SRAP, AFLP, SSR, ISSR and RAPD. As suggested by the authors, low polymorphism is often observed in watermelon cultivars, combining several marker systems is necessary to construct a high density genetic map covering the whole genome. SRAP markers have been used to construct genetic maps in a wide range of plant species. In Dendrobium plants that are used as Chinese herbs, Xue et al., (2010) constructed two genetic maps in two Dendrobium species, *D. officinale* and *D. hercoglossum* with a double pseudo testcross strategy using SRAP and RAPD methods. In root plants, Chen et al., (2010) constructed a genetic map in an F1 population derived from an inter specific cross in cassava by combining AFLP, SSR, SRAP and expressed sequence tag (EST)-SSR markers. In total, they assembled 355 markers into 18 linkage groups covering a genetic distance of 1,707.9 c M, which served as a foundation for QTL mapping in this species. In grass species, Xie et al.,(2011) used SSR and SRAP markers to construct two genetic maps of male and female parental lines respectively in diploid orchard

grass (*Dactylis glomerata* L.) using a pseudo-test cross strategy. In total, they assembled 164 SSR markers and 108 SRAP markers on these two genetic maps. In a fruit tree, Luohanguo (*Siraitia grosvenorii* C. Jeffrey), Liu et al., (2011) used SRAP and ISSR markers to assemble a genetic map consisting of 170 SRAP markers and 29 ISSRs in 25 linkage groups. In a fiber crop, Chen et al., (2011) use SRAP, ISSR and RAPD markers to construct a genetic map in kenaf (*Hibiscus cannabinus* L.) that is one of the most economically important fiber crops globally.

SRAP technology is an effective molecular marker system to analyze qualitative and quantitative resistance to plant diseases. In general, qualitative and quantitative resistances are conferred by oligogenic or multi genic loci, respectively. In canola, blackleg is a major disease and qualitative resistance is available. We used the previously described ultra dense genetic map to tag resistance genes to blackleg in *B. napus.* After screening 384 SRAP primer combinations, we identified two SRAP markers that were linked to a blackleg resistance gene. By compared the linked SRAP markers with the molecular markers on the ultra dense *B. napus* genetic map that was constructed with another mapping population, we found that one SRAP marker corresponded to a SRAP marker onN10. Therefore we took the flanking SRAP markers of the mapped resistance locus on N10 and identified other SRAP markers on the genetic map that were also polymorphic in the mapping population of the blackleg resistance gene. Eventually, further analysis allowed us to identify two blackleg resistance genes in the region where one resistance gene was suggested by other researchers (Long et al., 2010).

In several reports, SRAP markers were used to map genes controlling resistance to plant diseases in several crop species. For instance, Yi et al., (2008) used SRAP, STS and SSR markers to tag a resistance gene (*Pm4b*) to powdery mildew in wheat. They tested 240 SRAP primer

combinations and identified two SRAP markers linked to the *Pm4b* gene. Eventually, they mapped the *Pm4b* gene on chromosome 2AL that was flanked by SRAP, STS and SSR markers. In another study on gene mapping in wheat, Chen et al., (2012) used SSR, SRAP and TRAP markers to tag a wheat strip rust resistance gene. Using 400 SSR, 315 pairs of SRAP primers, and 40 pairs of TRAP primers to screen F1, F2 and BC1 mapping populations, they constructed a fine map flanking the resistance gene locus on chromosome arm 2AS and suggested that the mapped resistance gene should be a novel one. In sesame, Zhang et al., (2010b) performed genetic diversity analysis using SRAP and SSR markers. They analyzed 404 landraces from a sesame collection in China. Using11 SRAP and 3 SSR 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 and SSR 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.

SRAP amplification is actually a small portion of all possible sampling of a genome. So SRAP can be used to produce a reduced genome samples when multiple SRAP reactions are pooled. As described previously, pooled SRAP produces can be directly sequenced using next generation sequencing technologies. When replacing genomic DNA with c DNA

samples, SRAP is adequate to perform gene expression profiling and also construct c DNA genetic maps.

SRAP molecular technology is very useful in plant breeding. In QTL mapping, common QTL for the same trait of interest can be effectively identified. Since SRAP has a high throughput feature, multiple mapping populations can be analyzed effectively to construct several genetic maps. In addition, the same set of SRAP primers allows detection of the same genetic loci, which can used to align several genetic maps. SRAP is effective and efficient in marker assisted selection in plant breeding since thousands of samples can be analyzed inexpensively. SRAP technology has been commonly used in analysis of genetic diversity of manyplantspecies. Currently, SRAP are in most crops, tree species, ornamental and medicinal plants.

CHAPTER THREE

MATERIALS AND METHODS

3.1 The Experimental Site (Shambat) description:

The study was conducted at 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 21º c 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, Ph 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 Land	Ethiopianvarietyintroduced by 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
$5_{(3)}$	Land race variety collected by ARC
Harba 15	Local variety collected by ARC
Tailandi	Introduced variety by ARC
J4	Introduced variety by ARC
17	
$13_{(3)}$	Land race variety collected by ARC
Kenana	Released variety by ARC Variety
Tagrib	Inbred line by ARC

ARC: Agricultural Research Corporation.

Abbreviation 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**

3.3 Land 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 holes 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 13/7/2013: the second on 15/7/2013, 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 growth and yield characters were measured after the plants at each plot reached physiological maturity as the following.

3.4.1 Plant height (cm):

It was measured from surface of the ground up to the last part in plant on ten randomly selected plants at each plot.

3.4.2 Height to first capsule (cm):

It was measured from surface of the ground up to the first capsule in the plant on ten randomly selected plants at each plot.

3.4.3 Stem diameter (cm):

It was measured as the averages after maturity take the stem after (5 cm) from the surface of the ground up.

3.4.4 Number of branches per plant:

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

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. as described by Gomez and Gomez (1984).

3.6Coefficient of variation (C.V):

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

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

3.7. Standard error (S.E):

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

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= $\sqrt{\frac{error\ mean\ squarea}{r}} \times t$

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) =$g^{s2}+e^{s2}$

Genotypic variance ($\delta^2 g$) = $\frac{M2-M1}{r}$

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 Extraction 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/Isomayl 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 highly significant differences at (P< 0.01) for height of first capsule, stem diameter and number of branches, and significant difference at (P< 0.05) for plant height.

4.1.1Plant height (cm):

The results showed that the highest value (143.51cm) was obtained by the genotype Carawy, while the lowest value (111.63cm) was scored for the genotype 17. The grand mean was (129.32cm) and the coefficient of variation was (9.55) %

(Table: 4-2).

4.1.2Height first capsule (cm):

The results showed that the highest value (92.83cm) was obtained by the genotype Alradom, while the lowest value (63.20cm) was scored for the genotype Tailandi, The grand mean was(76.99cm) and the coefficient of variation was (12.47) %

(Table: 4-2).

4.1.3 Stem diameter (cm):

The results demonstrate that genotypes Abraway exhibited the highest value (6.89cm), whereas the lowest value (3.55cm) was scored by the genotype 13. The grand mean was (4.74cm) and the coefficient of variation was (13.02) %

(Table: 4-2).

4.1.4Number of branches:

The genotype Abraway obtained the highest number of branches (7.9333),where genotype 13 attained the smallest number of branches (1.7333) the grand mean was (4.9473) and the coefficient of variation was (29.45) % (Table: 4-2).

Table (4.1). Mean squares from the individual analysis of growth 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
Plant height (cm)	141.866	298.208*	152.491
Height of first capsule (cm)	355.121	277.521**	92.221
Stem diameter (cm)	0.94861	2.16414**	0.38007
No. of branches	3.19018	9.08334**	2.12282

**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	Plant height(cm)	Height of first capsule (cm)	Stem diameter (cm)	No .of branches
Abraway	136.45	81.950	6.8933	7.9333
Om shugaira	138.25	82.607	5.5467	6.6867
Alsodana	133.51	85.507	4.9267	6.2667
Danab Algamal	137.00	87.333	5.2200	6.6667
Kenana	133.06	82.200	4.4733	5.4667
Alradom	142.33	92.833	5.8367	5.9333
13	121.53	73.713	3.5467	1.7333
Caraway	143.51	87.113	4.7400	6.6000
Bash	126.37	67.767	4.1267	2.3333
J4	133.00	84.133	5.1467	4.8000
Harba15	112.53	63.667	4.0200	4.5333
High land	126.72	77.700	4.7600	4.4000
Tailandi	112.23	63.200	4.0267	3.3333
17	111.95	68.767	4.2467	3.6000
Solaimani	125.60	66.700	3.7000	4.5000
5	130.80	79.000	4.2133	3.0167
Tgarib	133.50	74.600	5.0867	6.3000
Grand Mean	129.32	76.988	4.7359	4.9473
C.V	9.55	12.47	13.02	29.45
SE	7.1295	5.5444	0.3559	0.8412
LSD	10.083	7.8410	0.5043	1.1896

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

The results showed that phenotypic variances values were higher than the genotypic variances for all growth characters (Table4-3). The highest value of phenotypic variance (450.699) was scored by the plant height. The lowest value (2.54421) was obtained by the stem diameter.

For genotypic variances the (61.76) were scored by the height of first capsule , The lowest value (0.59469) was obtained by the stem diameter. Respectively (Table 4-3).

4.3 Phenotypic (PCV %) and Genotypic (GCV %) coefficient and Heritability :

The results showed that phenotypic coefficient of variation had higher values than the genotypic coefficient of variation for all studied traits. The highest values of phenotypic coefficient of variation(219.148-153.08%) were scored by height first capsule and plant height the lowest values (150.502-73.295%) were scored by number of branches and stem diameter respectively (Tabl4e 4-3).The highest value of genotypic coefficients of variation (146.02-80.220%) were obtained by number of branches and height first capsule respectively, while the lowest values genotypic coefficient (37.651-35.435) were scored by plant height and stem diameter respectively(Table 4-3).

The highest value of heritability (23.374-) was obtained by stem diameter respectively. While the lowest values of heritability (10.776) were scored by plant height respectively (Table 4-3).

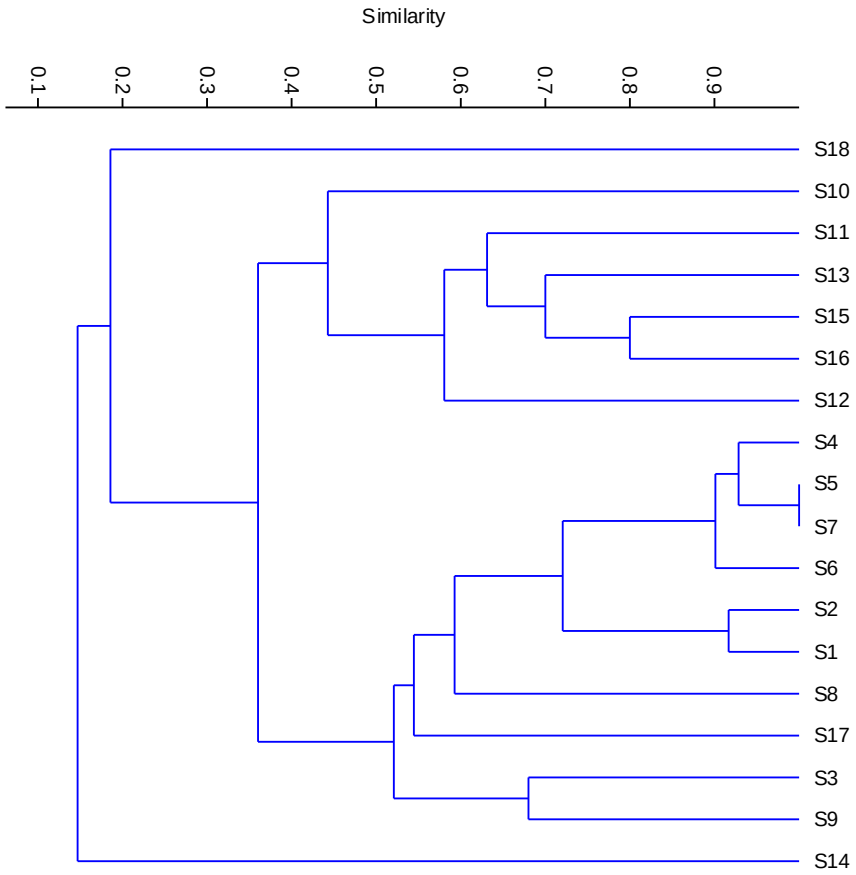
Table (4-3) Phenotypic (PCV %) and Genotypic coefficient of variation (GCV %), phenotypic and genotypic variance for the deferent characters studied on seventeen genotypes of sesame evaluated at Shambat in season

2014 - 2015.

Characters	Phenotypic variance e	Genotypic Variance	Genotypic Coefficient of Variation (GCV)%	Phenotypic Coefficient of Variation (PCV)%	Heritability (h)%
Plant height	450.699	48.57	37.651	153.08	10.776
Height.F.capsule	369.742	61.76	80.220	219.148	16.703
Stem diameter	2.54421	0.59469	35.435	73.295	23.374
No. of branches	11.2062	2.32017	146.02	150.502	20.704

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 all of growth traits 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).

5.1.1 Plant Height (cm):

In this study the genotype Caraway attained the highest value of the plant height of (143.51) the results were differ with how attained the range from 191.47 were reported by (Umar-Shaba, 2012).

5.1.2 Height first capsule (cm):

In this study the genotype Alradom attained the highest value of the height first capsule of (92.83).

5.1.3 Stem diameter (cm):

In this study the genotype Abraway attained the highest value of the stem diameter of (6.89) the results were in differ with how attained the range from 7.15were reported by (Umar-Shaba, 2012).

5.1.4 Number of branches per plant:

In this study the genotype Abraway attained the highest value of the Number of branches per plant of (7.93) the results were in differ with how attained the rang from (7.10)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 (PCV %):

The phenotypic coefficient of variation GCV all traits showed highest value than the genotypic variances. Similar finding 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.

CHAPTER SIX

CONCLUSIONS

Based on the results obtained for this study, it could be concluded as the following:

1. Variability obtained in this research between sesame genotypes for growth 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 growth 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.

REFERENCES

Abdlhafiz.m.E. (2001). Effect of partially acidulated phosphate rocks and triple super phosphate and their combination on growth mineral composition and yield of wheat. Ph.D. Thesis, Sudan University of Science and Technology.

Adam H.s (2002). The agricultural climatology (in Arabic).Second edition university of gazina press, wed madain, Sudan, pllg.

Bapu, J.R.K.(1992). Genotypic association and path analysis in f3 generation of rice crosses. Madras Agriculture Jour.76:619-623.

Burden, D. (2005). Sesame profile mhtm:file://C:/Document and setting / user1/crop profiles.mht(15/1/08).

FAO.(2008).food and Agriculture Organization of the UnitedNations. FAOSTAT.http:// faosta.fao.org/site/567/default.aspx#ancor.

Gandhi, A.P (2009).Simplified process for the production of sesame seed (*Sesamum indicum* L.)Butter and its nutritional profile.As.J.Food Ag-Ind.2 (1):24-27.

Ibrahim, S.E (2010).Variability of seed Yield, Yield Components and some Quality parameters in some Sesame (*Sesamum indicum*.) Genotypes. Ph.D. Thesis Faculty of Agriculture, University of Khartoum.

Khidir, M.O.(1997).Oil seed Crops in the Sudan(In Arabic).Khartoum University Press, 2nd Edition, Khartoum ,Sudan.

Narayanan, R. and S. Murugan (2010). Studies on Variability and Heritability in Sesame.

Ukaan S. I. (2012). Yield evaluation of 13 sesame (*Sesamum indicum* L.) accessions in the derived savannah agro-ecology of south-eastern Nigeria.

Umar-Shaba, Y. G (2012). Influence of poultry manure application on growth and yield performance of accessions of sesame (*Sesamum indicum* L.) in a derived savanna transition zone of South eastern Nigeria .

Chen, M., Wei, C., Qi, J., Chen, X., Su, J., Li, A., Tao, A., & Wu, W. (2011). Genetic linkage map construction for kenaf using SRAP, ISSR and RAPD markers. *Plant Breed.* , 130, 679-687

Sabesan , T . Suresh , R . and Saravanan , K . (2009). Genetic variability and correlation for yield and grain quality characters of rice grown in coastal saline low land of Tamilnadu. Electronic J . plant Breed . 1:56-59.

Chen, S., Chen, G., Chen, H., Wei, Y., Li, W., Liu, Y., Liu, D., Lan, X., & Zheng, Y.(2012). Mapping stripe rust resistance gene YrSph derived from *Tritium sphaeroco*

Gulsen, O., Uzun, A., Canan, I., Seday, U., & Canihos, E. (2010). A new citrus linkage map based on SRAP, SSR, ISSR, POGP, RGA and RAPD markers. *Euphytica* , 173, 265-277. Perc. with SSR, SRAP, and TRAP markers. *Euphytica* , 185, 19-26.

Liu, L., , X., Wei, J., Qin, J., & Mo, C. (2011). The first genetic linkage map of Luohan-guo (Siraitia grosvenorii) based on ISSR and SRAP markers. Genome, 54, 19-25.

Long, Y., Wang, Z., Sun, Z., Fernando, D. W. G., Mc Vetty, P. B. E., & Li, G. (2010). Identification of two blackleg resistance genes and fine mapping of one of these two genes in a *Brassica napus*

canola cultivar ‘Surpass 400’. *Theor Appl Genet.* , 122, 1223-1231.

Levi, A., Thomas, C. E., Trebitsh, T., Salman, A., King, J., Karalius, J., Newman, M.,Reddy, O. U. K., Xu, Y., & Zhang, X. (2006). An extended linkage map for waterme-lon based on SRAP, AFLP, SSR, ISSR, and RAPD markers. J Am Soc Hortic Sci. , 131,393-402.

Xie, W., Zhang, X., Cai, H., Huang, L., Peng, Y., & , X. (2011). Genetic maps of SSR and SRAP markers in diploid orchard grass (Dactylis glomerata L.) using the pseudotestcross strategy. Genome, 54, 212-221.

Xue, D., Feng, S., Zhao, H., Jiang, H., Shen, B., Shi, N., Lu, J., Liu, J., & Wang, H. (2010). The linkage maps of Dendrobium species based on RAPD and SRAP markers. Journal of Genetics and Genomics, 37, 197-204.

Yi, Y., Liu, H., Huang, X., An, L., Wang, F., & Wang, X. (2008). Development of molecular markers linked to the wheat powdery mildew resistance gene Pm4b and marker validation for molecular breeding. Plant Breed. , 127, 116-120.

Zhang, W., Pan, J., He, H., Zhang, C., Li, Z., Zhao, J., Yuan, X., Zhu, L., Huang, S., &Cai, R. (2012). Construction of a high density integrated genetic map for cucumber (Cucumis sativusL.) Theor Appl Genet. , 124, 249-259.