



بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

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Genetic Diversity of Sesame Genotypes using Agro- morphological, Abiotic Stresses Effects and Genomics Studies

A thesis Submitted in Fulfillment of the Requirements
for the Degree of Ph.D. in Plant Biotechnology

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Shendi- Dec. 2023

بِسْمِ اللّٰهِ الرَّحْمٰنِ الرَّحِیْمِ

(وَهُوَ الَّذِي أَنْزَلَ مِنَ السَّمَاءِ مَاءً فَأَخْرَجْنَا بِهِ نَبَاتَ كُلِّ شَيْءٍ فَأَخْرَجْنَا مِنْهُ خَضِرًا نُّخْرِجُ مِنْهُ حَبًّا مُتَرَاكِبًا وَمِنَ النَّخْلِ مِنْ طَلْعِهَا قِنْوَانٌ دَانِيَةٌ وَجَنَّاتٍ مِّنْ أَعْنَابٍ وَالزَّيْتُونَ وَالرُّمَّانَ مُتَشَابِهًا وَغَيْرَ مُتَشَابِهٍ انظُرُوا إِلَى ثَمَرِهِ إِذَا أَثْمَرَ وَيَنْعِهِ إِنَّ فِي ذَلِكُمْ لَآيَاتٍ لِّقَوْمٍ يُؤْمِنُونَ).

الانعام (99)

صدق الله العظيم

Dedication

I dedicate this project to God Almighty my creator, my strong pillar, my source of inspiration, wisdom, knowledge and understanding. He has been the source of my strength throughout this program and on His wings only have I soared. I also dedicate this work to my family and many friends. A special feeling of gratitude to my loving parents, whose words of encouragement and push for tenacity ring in my ears.

This work is also dedicated to all aspiring students and future researchers.

ACKNOWLEDGEMENT

Firstly, this research work has been a great journey for me and has helped me to understand an area of work that is vast and wonderful. It has been completed with years of hard work and dedication and would not have been possible if not for the blessing and guidance I have received from a number of people.

For this I am particularly indebted to my supervisor **prof. Tag Alsir Hassan Mohamed Ahmed** who has not only been a guide but also a mentor whom I could depend on for advice, encouragement, and support. In particular, I would like to thank my supervisor **Dr. Mai Abdelmoez Abdelmoghny Khalil Allam** – from the National Research Center - Cairo – Egypt the partner of the research who provided information and access to knowledge bases that has helped me greatly. A special mention of thanks to my co-supervisor **Dr. Seedahmed Ahmed Mohamed** who have helped me review and also provided guidance at crucial times during the research.

I also greatly appreciate the contributions of my friends and colleagues from the Faculty of Science and Technology and Shendi university who gave me the opportunity to conduct this work and facilitated the necessary resources to conduct this research.

My sincere grateful thank to the national research center Shendi university staff for their cooperation.

I wish to express my sincere gratefulness to my lovely family for their support. Without them I would not be able to reach this step in my scientific career.

ABSTRACT

The experiments were conducted of the faculty of science and technology, Shendi university Sudan. To study the genetic diversity of sesame (*Sesamium indicium*) on agro morphological effect of drought and salinity on seed germination and molecular markers (ISSR). Afield experiment was conducted to study the agronomical differences among 100 sesame genotypes in complete randomized block design in three replicates. Laboratory experiments were conducted to evaluate the difference among 60 genotypes tested for germination shoot and root length under drought stress (0, 6 and 9) bar and salinity stress (0,60 and 80) mM. The genetic diversity of the sixty genotypes was study by molecular marker (ISSR). Significant differences were observed between the genotypes regarding plant height, number of branches per plant and number of capsules per plant. The abiotic stress significantly affects seed germination, seedling shoot and root length increasing the concentration of PEG (6000) from (0,6 and 9) bar and increasing the concentration of NACL (0.60 and 80) mM significantly decrease the parameter test. The stander germination test using germinator curve software showed a significant decrease in the frequency of TG, NS, AB, SL, RL, RS, T10, T50 and AUC with increasing either drought or salinity concentration as indicated by the histogram and Box plot. Molecular DNA markers analysis with 10 primer showed 100% polymorphism. With 10 bands of 10 primers. The PIC % and the marker index vary between the primers depend on the number of bands.

المستخلص

العنوان: دراسة التنوع الجيني والتأثير المورفولوجي لاجهادات الجفاف والملوحة لنبات السمسم (*Sesamium indicium*).

أجريت الدراسة بكلية العلوم والتقانة ومعمل الأبحاث المركزي جامعة شندي السودان , دراسة التنوع الجيني والتأثير المورفولوجي لاجهادات الجفاف والملوحة لنبات السمسم (*Sesamium indicium*). أجريت التجربة الحقلية بالمزرعة التجريبية بهيئة البحوث الزراعية شندی لدراسة الفروق المورفولوجية بين 100 صنف وراثي للسمسم السوداني في تصميم القطاعات العشوائية الكاملة في ثلاث مكررات. وتم اختيار 60 صنف وراثي لاجراء التجارب المعملية لدراسة نمو بذور السمسم تحت الاجهاد البيئي (طول الساق والجذر). تمت دراسة تأثير إجهاد الملوحة بتراكيز (0, 60 , 80) ملي مولار من كلوريد الصوديوم ودراسة تأثير إجهاد الجفاف بتراكيز (0 ، 6 ، 9) بار من البولي إيثيلين جليكول. أجريت التجارب بالتصميم العشوائي الكامل بثلاثة مكررات. وتمت دراسة التنوع الجيني للأنواع الجينية الستين بواسطة (ISSR). باستخدام 10 برايمر. ثم أثبتت الدراسة وجود فروقات معنوية بين الطرز الوراثية فيما يتعلق بطول النبات وعدد الأفرع لكل نبات وعدد الكيسولات لكل نبات. اثبتت الدراسة ان الاجهاد البيئي ادى الي فروقات معنوية في انبات البذور وطول الساق والجذر في البادرات . ادت الزيادة في تراكيز الاملاح الي نقيصة معنوية في انبات البذور وطول الساق والجذر في البادرات. كما أظهر اختبار الإنبات القياسي باستخدام برنامج منحنى الإنبات انخفاضاً معنوياً في تواتر TG و NS و AB و SL و RL و RS و T10 و T50 و AUC مع زيادة تركيز الجفاف أو الملوحة كما هو موضح في الرسم البياني ومخطط الصندوق. وأظهر تحليل الحمض النووي الجزئي باستخدام 10 برايمر تباين وراثي بنسبة 100%. تختلف PIC% ومؤشر العلامة بين البرايمر المختلفة وتعتمد على عدد النطاقات.

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Abbreviations

List of abbreviations

Abn	Abnormal Seedlings
CT	Control seeds
DNA	De-oxy ribose Nucleic Acid
NS	Normal Seedlings
RL	Root Length
RSH	Root/Shoot ratio
SD	Standard deviation
SGT	Standard Germination Test
SHL	Shoot Length
ISSR	Inter Simple Sequence Repeats
T10	Time required to reach 10% germination
T50	Time required to reach 50% germination
TG	Total Germination
ROS	reactive oxygen species

Chapter one

Introduction & Objectives

Chapter one

1.1 Background

Sesame (*Sesamum indicum L.*) is one of the world most important and oldest known oil seed crops. Its cultivation dated as far as 1500 BC in the Middle East, Asia and Africa (Ali, Yasumoto, and Seki-Katsuta 2007). Sesame is the earliest oilseed plant to be used by humans, adapted to tropical climates and with low water demand, sesame is a good cultivation option for semi-arid regions as it is rich in proteins and can provide an alternative income source, especially for small and medium farmers. Sesame has moderate salt tolerance, but will not grow under flooded conditions. Selection and growing of salt tolerant food and forage crops could increase productivity and production of crops in marginalized salt affected land. (Mamo, *et al.* 2021).

Sesamum indicum L. is an annual plant of pedaliaceae family considered to be the oldest of the oilseed plants and has been under cultivation in Asia for over 5000 years. Sesame is cultivated on seven million ha worldwide; India and China are the world's largest producers of sesame, followed by the areas found in developing countries Myanmar, Sudan, Uganda, Ethiopia, Nigeria, Tanzania, Pakistan and Paraguay. Sesame and its components serve as viable natural sources of antioxidants for food and non-food applications. High level of PUFAs in sesame oil is claimed to reduce blood cholesterol, high blood pressure and play an important role in preventing atherosclerosis, heart diseases and cancers.(Sharma, *et al.* 2014).

Sesame is important oilseed crop of tropical and sub-tropical region, renowned for its high oil content (up to 60% oil), hence sesame is known as the king of oil seeds. Sesame seed oil contains 83% - 90% unsaturated fatty

acids, 20% proteins and various minor nutrients such as vitamins and minerals, large amount of characteristic lignans, such as sesamin, sesamol, sesamolol and tocopherols. Sesame seeds with high amounts of nutritional components are consumed as a traditional health food for its specific antihypertensive effect, anti-carcinogenic, anti-inflammatory and antioxidative activity. Therefore, serious efforts are necessary for selecting varieties of good quality and high adaptive potential to the diverse climatic situations. There should be effective strategies adapted to produce climate ready sesame variety using modern biotechnological approach.(Pathak, *et al.* 2017).

White sesame (*Sesamum indicum* L.) flour, an edible residue resulting from seed oil extraction, may become a novel alternative of healthy products due to its high polyphenols content—compounds with many beneficial effects for human health.(Sabatino, *et al.* 2021).

Sesame is an important crop in West and Central Africa playing a role of an alternative cash crop for small holders. However, sesame productivity is highly impaired by drought, Plant growth, development, productivity, and resistance to climatic stresses are currently the major topics of interest for agriculture and plant-based biotechnologies. Both biotic (e.g., phytopathogens) and a biotic stress (e.g., drought, salinity, flood, storm, and extreme temperatures) cause enormous losses in agricultural production (Fraire-Velázquez and Balderas-Hernández, 2013).

They produced higher biomass and had higher ability to maintain seed quality under drought stress compared with the sensitive genotypes. Strong accumulation (~200% ratio stress/control) of biochemical markers including superoxide dismutase, ascorbate peroxidase, catalase and proline could be

regarded as an important indicator for selecting drought resistant genotypes.(Dossa, *et al.* 2017).

Salinity is the accumulation of salt often dominated by sodium chloride in soil and water to the level that has negative impact on plant. Sesame (*Sesamum indicum L.*) is an annual crop with height ranging from 50 to 100cm tall. It is one of the important oil crops and ranked 9th among the top thirteen oil seed crop .(Muhammad, *et al.* 2018). Salinization is a global environmental problem. It is particularly prevalent in Africa in areas with a low rainfall trend such as the Senegalese groundnut basin where 20% of the land is affected. It reduces global food production by more than 10%. In Senegal, sesame (*Sesamum indicum L.*) moderately tolerant to drought and salinity is increasingly cultivated. It is an alternative to fight poverty in rural areas and allows the revaluation of salty land.(Dangue, *et al.* 2022).

One of the molecular markers to analyze plant genetic diversity is Inter Simple Sequence Repeats (ISSR). ISSR involved using micro-satellite sequence order that spread across the genome as primers in a polymerase chain reaction to produce multi-locus markers. ISSR markers could detect the level of genetic diversity and group sesame collections.(Anggraeni, *et al.* 2022).

Molecular marker based genetic diversity assessment of sesame cultivars is of great value to assist parental line selection and breeding strategy blueprint (Wu, *et al.* 2014). Inter simple sequence repeat (ISSR) markers are powerful tools, which can be utilized to access the variation around the diverse microsatellite regions that are dispersed throughout all genomes (Weldemichael, *et al.* 2019). ISSR or anchored SSR primers are complementary to genomic microsatellites (one to four nucleotides occurring in tandem repeats) and contain short oligo-nucleotide ‘anchor’ sequences that

ensure the primers anneal to either the 5' to 3' end of the genome repeat.(Weldemichael, *et al.* 2019). ISSR marker is more reproducible and cost effective for researchers in developing countries like Ethiopia. The technique does not need any prior information about DNA sequence and overcomes many of the technical limitations of RAPD and AFLP. (Abate, *et al.* 2015).

1.2 Problem statement

Conservation of Arabian important genetic resources of sesame, physiological analysis and morphological and genetic characterization, to maximize translation of research outputs into commercial practice and to support the wider decision-making that the sustainable use of sesame crop on salinized lands and genetic mapping of loci and genes associated with abiotic stresses on sesame seeds.

1.3 Objectives

General objective:

To assess the pattern and extent of genetic variability in sesame germplasm collection of Sudan using agro-morphological and physiological traits and molecular markers to generate information used in future.

Specific objectives:

1. To determine the level of morphological variation and association of traits in sesame genotypes.
2. Study the growth performance and physiological traits under abiotic stresses (salinity and drought).

3. Molecular characterization of sesame genome (Genotyping) using one of the genetic diversity is Inter Simple Sequence Repeats (ISSR).

Chapter two

Agro - Morphological Diversity of Sesame Genotypes

Chapter two

2.1 Literature review

2.1.1 Sesame (*Sesamum indicum* L.)

Sesamum indicum L. is an annual plant of pedaliaceae family considered to be the oldest of the oilseed plants and has been under cultivation in Asia for over 5000 years. Sesame is cultivated on seven million ha worldwide; India and China are the world's largest producers of sesame, followed by the areas found in developing countries Myanmar, Sudan, Uganda, Ethiopia, Nigeria, Tanzania, Pakistan and Paraguay. (Sharma *et al.* 2014).

Sharma *et al.* (2014) was reported the white sesame cultivar content low protein, sesame oil is claimed to reduce blood cholesterol, high blood pressure and play an important role in preventing atherosclerosis, heart diseases and cancers.

Sesame is used as active ingredients in antiseptics, bactericides, viricides, disinfectants, moth repellants, and anti-tubercular agents. In spite of being a good source of “healthy oil” in terms of presence of high amounts of PUFA and high antioxidant content, it is not grown on a large extent due to very poor yields. Therefore, serious efforts are necessary for selecting varieties of good quality and high adaptive potential to the diverse climatic situations. There should be effective strategies adapted to produce climate ready sesame variety using modern biotechnological approach (Pathak *et al.* 2017).

White sesame (*Sesamum indicum* L.) flour, an edible residue resulting from seed oil extraction, may become a novel alternative of healthy products due to its high polyphenols c

content—compounds with many beneficial effects for human health (Sabatino *et al.* 2021).

Sesame is an important crop in West and Central Africa playing a role of an alternative cash crop for smallholders. However, sesame productivity is highly impaired by drought. The resistant genotypes exhibited both avoidance and tolerance features including increase of the root system, reduced water loss, highest activity of antioxidant enzymes and accumulation of proline. They produced higher biomass and had higher ability to maintain seed quality under drought stress compared with the sensitive genotypes. For future research towards developing new varieties with improved drought resistance in West and Central Africa (Dossa, *et al.* 2017).

Sesame is the earliest oilseed plant to be used by humans, adapted to tropical climates and with low water demand, sesame is a good cultivation option for semi-arid regions as it is rich in proteins and can provide an alternative income source, especially for small and medium farmers (Mamo *et al.* 2021).

Sesame is one of the most important oil crops widely grown in different parts of the world. For many centuries, sesame seeds have been used as a source of oil, protein, vitamins, and minerals for human and animal nutrition (Biosci, *et al.* 2014). Sesame oil is a very stable one due to its content of such antioxidants as sesamin, sesamol, and sesamol (Suja *et al.* 2004). The seed is not only rich in oil (42–45%) but also in proteins (20%) and carbohydrates (14–20%). The micronutrient content of sesame seeds generally follows the order Fe>Cu>Zn>Mn (Suresh *et al.* 2013). Iran is mostly characterized by arid and semi-arid climates. Drought stress in this region is, therefore, one of the most important environmental factors reducing the growth and yield of many crops (Reddy, *et al.* 2004). Having an average annual precipitation of 250 mm,

Iran receives less than one third of the global average precipitation (750 mm). To this must be added the problem of uneven distribution of precipitation so that water shortage is a common feature of both irrigated and rain-feed farms in Iran (Khorasanizade 2012). Plant responses to abiotic stresses, especially drought, include the production of reactive oxygen species (ROS) and other toxic substances (Xiong, *et al.* 2002). That cause fatty acid peroxidation and damage to cell membranes, which in turn create small hydrocarbon segments such as Malondialdehyde (MDA) (Moussa and Abdel-Aziz 2008). Increased Malondialdehyde content is a sign of drought impact on plant cells. Over the last decade, the production of sesame seeds has doubled, showing the increasing interest on this crop (Dossa, *et al.* 2017). Sesame is especially significant in the arid and semi-arid regions where the cultivation of several major crops including maize, cotton, etc., becomes problematic. In fact, salinity and water deficit have a strong influence on agricultural development in these regions (Ramirez *et al.* 2005). The diversity in sesame responses to salt and drought stresses are controlled by genetic factors. Therefore, understanding the genetic components for salt and drought tolerances by identifying the genetic loci and the candidate genes associated with these traits would be an economical, feasible and efficient way to accelerate the progress of abiotic tolerance breeding in sesame (Dossa, *et al.* 2017). Seed germination is one the most sensitive stages of sesame growth cycle (Boureima *et al.* 2011). Accordingly, detecting some genetic variants associated with sesame tolerance to drought and salinity at the germination stage will be a major asset for sesame cultivation in arid and semi-arid regions.

2.1.2 Sesame in Sudan

Sesame is one of the important cash crops in the Sudan and is grown under rain fed conditions by traditional or mechanized farming, semi- commercial and commercial farmers. It is grown mainly in the sandy soils of Northern Kordofan and central clay plain, especially in Al-Gedarif and Al-Damazin areas. It is also grown in Southern Kordofan and Southern Darfur and on a small scale in the southern region.(Khidir 2003) In the Sudan, sesame is one of the important oil crops, coming third in production area after sorghum and millet. It draws its importance from its use as a food crop, a raw material for industry, feed for livestock and as a leading export crop (Abdel Rahman, 2008) Sudan is the third largest producer of sesame in the world, after India and China. Nevertheless, it is considered the main world exporter of sesame seeds (Maryoud, *et al.* 2008) Sudan ranks second in the volume of sesame exports and accounts for 80% and 40% of all cultivated sesame area in the Arab world and African continent, respectively.

In the recent past the country witnessed an expansion in area planted to sesame. The area under sesame cultivation is currently estimated at about 4,938,238 feddans. (Abdalla Jumalla 2017) 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 Sesame seed production in Sudan is estimated at about 450,000 MTs and is grown under rain-fed conditions by subsistence, semi- commercial and commercial farmers. It contributes to Sudan's export trade and earnings. This cultivation range extending from the semi-arid tropics to sub-tropic temperate regions has led to a wide diversity of genotypes (Sogut. 2008).

The main differences in the yield variation in terms of morphological properties and chemical composition of sesame cultivars have been attributed to the lack of new cultivars for high yield, early maturity, non-shattering and wide adaptation (Baydar 2005). In addition to differences in morphological properties, protein content, oil content and fatty acid compositions may vary considerably between genotypes under different environmental conditions (Sogut 2008).

There are three wild relatives of sesame recognized in Sudan including *S. alatum*, *S. latifolium* and *S. angustifolium*. The existing wide variability in cultivated landraces and the wide spread of wild types makes Sudan an important location for sesame genetic diversity. Collection efforts between 1999 and 2008 have resulted in more than 300 accessions from areas in eastern, western and central Sudan including both cultivated and wild material with different characteristics especially seed color (Nahar 2009). Genotypes vary in their adaptability to different growing conditions. The ability of sesame yield is determined by different yield components, all of them are substantially influenced by environmental conditions and agronomic practices (Caliskan *et al.* 2004).

2.1.3 Botanical description

Sesame (*Sesamum indicum* L.) Is flowering plant belongs to family Pedaliaceae, genus sesamum, it is one of the earliest domesticated crops (Bedigian, 2010). It is mainly planted in tropical and subtropical regions in Asia, Africa, and South America (Ashri and Janick 2010). It is extensively cultivated in the tropics and temperate zone of the world. India, China, Burma, Sudan and Mexico are the largest producers (Bennett and Condé, 2003).

Sesame is deep-rooted, which can reach up to 990 cm in length and will search nutrient from below most crop zones (Grichar, *et al.* 2011). The long taproot contains many lateral roots. In the early maturing varieties, the root system is poorly developed, but late maturing varieties have a well-developed root system. *S. indicum* is an erect annual plant that can grow to a height of 1.0 to 2.0 meters depending on the cultivar and the growing conditions (Bennett and Condé, 2003). However, varieties that are 1.0 to 1.4 m high are more common. Its erect stem is usually square. It bears elongated oval leaves on a stem which can be branched or un-branched, depending on the variety. The stem is covered with short, soft hairs. Sesame is characterized by opposite leaves and bell-shaped flower. The plant bears its flowers in the axils of the leaves. The color of the corolla varies from white to purple. The fruiting shape of sesame is a capsule, often called pods. They have divided sections like a cotton bowl. Varieties have a single capsule or triple capsules per leaf axil (Grichar *et al.* 2011) The seed is produced in capsules with about 70 seeds per capsule. Sesame seed can be black, brown or white in color and contains about 50% oil and 20% protein (Bennett and Condé, 2003). The blossoming and ripening phases take place over several weeks, starting at the bottom of the plant and progressing upwards (Augstburger *et al.* 2002). Physiological maturity normally occurs 95-110 days after planting for early types and up to 150 days for late types. Physiological maturity is when 75% of the capsules on the main stem have mature seeds or when three-fourth of the stem turns yellow (Abdalla Jumalla 2017).

2.1.4 Climatic conditions

Sesame is an important oilseed crop successfully grown in Tropical and subtropical climates from 25°N to 25°S (Abdalla Jumalla 2017) It has a deep tap root system and ability to set seeds under high temperature that enable it to grow well in different agro ecological zones, including the arid and semi-arid zones. Sesame needs a high temperature, the optimum range for growth, blossoms and fruit ripeness is 26-30°C (Augstburger *et al.* 2002). Low temperature at flowering can result in the production of sterile pollen or premature flower fall. Pollination and the formation of capsules are inhibited during heat wave periods above 40°C. Depending on the climate, sesame can be cultivated at altitudes up to 1600 m (Augstburger *et al.* 2002). Once established, sesame is capable of withstanding a higher degree of water stress than any other cultivated plants. However, it requires adequate moisture during germination and early growth for reasonable yields, Sesame is highly susceptible to water-logging and can therefore only thrive during moderate rainfall or when irrigation is carefully controlled in drier regions. Due to its tap roots, the plant is highly resistant to drought and can provide good harvests even when only stored soil water is available (Augstburger *et al.* 2002). Sesame is basically a short-day plant and with a ten-hour day, will normally flower in forty-two and forty-five days, but many varieties have become locally adapted to various light periods, Sesame needs long periods of sunshine and is generally a short day plant whereby varieties exist which are unaffected by the length of the day. Sesame is sensitive to strong winds when the main stem is fully grown. Tall varieties should not be planted in regions, which have strong winds during the harvesting season (Augstburger *et al.* 2002).

2.1.5 Production and productivity

A total of 6.5 million tons of sesame seed was produced on 9.42 million ha worldwide (FAOSSTAT 2022). Yields are not high, probably averaging less than 400 seed per Fadden, but this is offset by the high oil content of up to 50 per cent. Myanmar, India and China are the world's largest producers followed by the areas found in developing countries, Sudan, Uganda, Ethiopia, Nigeria, Tanzania, Pakistan and Paraguay. Higher productivity in sesame crop can be achieved through a combination of an ideal variety associated with proper environment and appropriate agronomic practices. Total area coverage in Ethiopia in 2013 was 283,000 ha with a production of 0.19 million tones (FAOSSTAT 2015). It showed a decline from the 2012 in yield per ha by 12.7%. Despite its nutritional, cultural and historic importance, the crop has low yielding capacity compared to other crop plants, mainly due to its low harvest index, susceptibility to diseases, seed shattering, indeterminate growth habit and asynchronous capsule ripening (Yol and Uzun 2012). The causes for low productivity are several and interdependent, in which the persistent use of traditional practices and unimproved local cultivars are at the forefront. Most of the local cultivars are the bushy type with a moderate yielding capacity but are less susceptible to bacterial blight disease and webworm attack (Amde 2005).

Sesame production and productivity are severely constrained by a lack of high-yielding and locally adapted varieties, susceptibility to capsule shattering and low seed retention, biotic and abiotic stresses, and a lack of modern production and pre- and post-harvest technologies.

The major sesame producing areas of Ethiopia are North-west Tigray (Humera and the surroundings), Amhara (North Gonder, Metemma and the

surroundings) and Oromia (East Wellela). These areas account for 91% of the total production in the country. However, average yield/ha in Ethiopia is quite low mainly due to lack of improved varieties and their packages for use by farmers. Therefore, current and future sesame genetic improvement programs should integrate yield- and quality-promoting traits, local adaptation, amenability to machine harvesting, and other industrially essential food and feed attributes for multiple utilities. ((Teklu, *et al* 2022). It is considered as one of the most ancient oilseeds crop known to mankind, it is also known as benn seed in Africa and sim-sim in East Africa. The genus has many species, and most of them are wild. Most wild species of the genus *Sesamum* are native to sub-Saharan Africa. *S. indicum*, the cultivated type originated in India (Bedigian, 2010).

Numerous wild relatives occur in Africa and a smaller number in India. It is widely naturalized in tropical regions around the world and is cultivated for its edible seeds, which grow in pods or "buns". Sesame. Is one of the oldest oilseed crops. Its cultivation history can be traced back to 3050-3500 BC in the Harappa Valley of the Indian subcontinent (Bajaj 2012). The genus has many species, and most are wild. Most wild species of the genus *Sesamum* are native to sub-Saharan Africa. *S. indicum*, the cultivated type originated in India (Bekele *et al.* 2017). 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(Nxele, *et al.* 2017).

Compared with the seeds of other main oil crops, e.g., rapeseed (*Brassica napus*),, soybean (*Glycine max*),, peanut (*Arachis hypogaea*) and olive (*Olea europaea*),,

sesame seeds not only have the highest oil content, but also are rich in proteins, vitamins, and specific antioxidants such as sesamin and sesamol. Because of its high oil quality and high nutritive value, sesame seed is regarded as ‘the queen of oil seeds’ and one of the best choices for health foods (Wang *et al.* 2014). (Mafakheri *et al.* 2010) Sesame cultivar (*Sesamum indicum* L.) has high nutritional value due the significant amounts of dietary fiber, protein, natural antioxidants, unsaturated fatty acids, vitamins, and mineral constituents present in its composition (Anilakumar *et al.* 2010).

2.1.5.1 Seed’s oil content

Sesame (*Sesamum indicum*) is an important oilseed crop belonging to the family Pedaliaceae. It is grown in Asia, Africa and parts of Latin America. The major sesame Producing countries are India, China, Thailand and Mexico. The yield per unit area in Bangladesh is lower than in other countries of the globe. Sesame yield in Bangladesh is 612 kg/ha, in India 322 kg/ha, in China 1089 kg/ha, in Mexico 556 kg/ha, in Thailand 615 kg/ha, in Egypt 1222 kg/ha (Abdalla Jumalla 2017).

Sesame oil has been reported as a source for biodiesel and found to give a product with fuel properties in parity with mineral diesel but with superior environmental performance(Ahmed, *et al.* 2012).The Seed oil content ranges from 45 to 63%, of which the principal fatty acids are oleic acid (18:1) (39.6%) and linoleic acid (18:2) (46.0%). Sesame seeds also contain a group of compounds called lignans, which have been reported to have many pharmacological properties, e.g. antioxidant activity, antihypertensive effects, it has high nutritive value and are used in numerous cuisines all over the world. The presence of some antioxidant (sesamum, sesamol or sesamol) makes the oil to be one of the most stable vegetable oils in the world. The

residue left after the extraction of oil is known as the oil seed cake which is used as livestock feed. Sesame is considered to have both nutritional and medicinal values. It is grown mainly for its seeds that contain approximately 35- 60% oil (Alyemeni, *et al.* 2011) and 25% protein. The seeds have many colors like red, white, black and yellow, depending upon the variety. Seed coat color is one of the most important agronomic traits of sesame. It is related to biochemical functions involved in protein and oil metabolism, antioxidant content, and disease resistance. The natural color of mature sesame seeds is diverse, varying from black to white through different intermediates such as gray, dark brown, brown, pale brown, yellow and dirty white (Bedigian, 2010). In general, pale-colored sesame seeds contain more oil than dark-colored ones (Abdalla Jumalla 2017).

Seed is one of the basic inputs in agricultural activities. It is part of plant that used for reproduction either vegetative or generative. The use of quality seed is one of the efforts in order to improve productivity. Sesame is a plant that contains 50-53% vegetable oil. Sesame plants propagation through seeds that produce generatively. This herb is said to be rich in polyunsaturated fatty acids and natural antioxidants, sesamin, sesamol and tocopherol homologues. Recent studies on the antioxidant and anti-carcinogenic activities of sesame seed have greatly increased its applications in health food products that assert for liver and heart protection and tumor prevention (Cheng *et al.* 2006).

The seed testing was to avoid planting seeds that do not have the ability to grow into expected plants. Seed germination testing is a method that provides to provide information for seed users about the seeds ability to grow into plants that have. The viability testing that usually used on sesame seeds is germination testing. The aim of germination testing is to detect seeds

germination viability under optimum condition. This research has purpose to determine the correlation between germination, vigor index, and maximum growth potential of sesame seeds. Seeds that used in this research were Sum berejo four varieties which have been stored for 6 and 7 years. (Hartati, *et al.* 2019)

2.1.6 Cultivation and conservation of sesame

Sesame is almost entirely cultivated under rain-fed in the Sudan. Poor management practices especially the practice of low seed rate as well as traditional cultivars are the main yield limiting factors in sesame farms of sandy dunes in north kordofan of Sudan. El Naim *et al.*, (2012) found that seed rate of 1.5 and 2.0 Kg ha⁻¹ were optimum for sesame cultivation under rain fed conditions in sandy dunes of North Kordofan state, Sudan. Row spacing of 25–75 cm is recommended for sesame crop in different countries(Negasa 2018). The establishment of an adequate plant density is critical for utilization of available growth factors such as water, light, nutrients and carbon dioxide and to maximize grain yield. Too wide spacing leads to low plant density per unit area and reduces ground cover, whereas too narrow spacing is related to intense competition between plants for growth factors (Ashri and Singh, 2006) On the other hand, the variation in plant density has been related to the variation in the number of capsules per plant, seed yield per plant and 1000-seed weight ,(Bakhshandeh 2006) and plant height, number of branches per plant and seed yield (Ngala, *et al.* 2013). Previous studies indicated that sesame cultivars are variable in their response to plant density. (El Naim, *et al.* 2010) found that increased plant population had the general tendency to increase seed yield (t/ha) and also showed that plant populations of approximately 166,666 and 249,999 plants ha⁻¹ were optimum

for sesame cultivation under rain fed conditions in North Kordofan state, Sudan.

2.1.7 Sesame fertilization

Sesame responds but poorly to applications of fertilizers. The application of fertilizers must be related to the growth stage of the plant, plant population, soil fertility and amount of soil moisture available. Sesame's nitrogen requirement can be fulfilled through organic sources, such as leguminous cover crops or animal manure. Phosphorous and potassium needs are not known exactly. If soils are acidic, pH should be brought up through liming (Terefe *et al.* 2012).

(Abdalla Jumalla 2017) found that the highest seed yield and yield components were obtained with 20 and 40 kg ha nitrogen and phosphorous rates, respectively. Mankar *et al.* (1995) showed that phosphorous application at the rate of 22 kg P ha significantly enhanced seed yield, seed oil content and seed protein content of sesame. (Olowe and Busari 2000) found that application of 60 kg N ha and 13.2 kg P ha produced significantly the highest number of capsules per plant, branches per plant, capsule weight per plant and grain yield per hectare.

Hossain *et al.* (2007) recorded the highest sesame seed yield with the application of 60 kg N ha⁻¹. Application of 44 kg N ha⁻¹ resulted in a marked increase in seed yield and yield components of sesame variety Shuak under conditions of Northern Sudan In order to study the effect of bio-fertilizers on sesame genotypes yield under drought stress, There is a significant interaction between fertilizer and stress and Nitrazhin fertilizer can reduce the effect of stress on the number of capsules.(Farokhian, *et al.* 2021).

2.2 Materials and methods

2.2.1 Field experiment

The experiment was carried out in a completely randomized design, in a $3 \times 3 \times 60$ factorial scheme, with three replicates, corresponding to sesame cultivars. The experiment was conducted in the demonstration in two consecutive seasons on a farm in Agricultural Research Corporation Shendi. To study the genetic variability, correlation of 100 genotype among sesame (*Sesamum indicium* L.) Under irrigation system. Shendi is located at latitude °E, longitude °N, and altitude m above sea level, within the semi-desert region the soil of the site. Randomized Complete Block Design was adopted with three replicates (RCBD) Land was immediately irrigated and subsequent irrigation was made every 7-10 days till the end of season.

2.2.2 Sample collection

Seeds of 100 sesames (*Sesamum indicium* L.) accessions were obtained from the Sudan National Gene Bank, Research Institute, Agricultural Plant Genetic Resources Conservation and Research Centre (table 2.1)

2.2.3 Numbers of branches per plant

The results were showed a significant difference ($P \geq 0.05$) in number of branches per plant between the sesame genotypes (table 2.3, fig. 2.2). The number of branches per plant was recorded in the range between 2 and 8 branch/plant. many researchers found a close range of number of branches per plant 4 to 8. The variations in number of branches per plant were detected in sesame genotypes in previous studies (El Naim *et al.* 2010).

(Terefe *et al.* 2012) reported that number of branching is affected by seed rate, rainfall, day length and variety. As observed in the plant height, the number of branches per plant also affected by origin of the seed source.

2.2.4 Land preparation and sowing

The land was ploughed, harrowed, leveled, ridged and divided into 100 ridges 60 cm between ridges. Five seeds of every genotype were sown in holes 10 holes per ridges 10 cm between holes. Sesame seedlings were thinned to two plants in each hole one week after sowing. Hand weeding was done manually four times during the growing season.

2.2.5 data collocation

At harvest (90 days) five plant were randomly selected from each ridge and the means of selected plants recorded for each parameter under test.

2.2.5.1 Plant height (cm)

Plant height was measured from the ground level to the tip of the plant.

2.2.5.2 Number of branches per plant

Number of branches was determined by counting the number of primary reproductive branches. Parameters were recorded.

2.2.5.3 Number of capsules per plant

This was determined by counting the number of capsules on the 5 sampled plants in each plot at harvest and the mean calculated and recorded.

2.2.6 Statistical analysis

The collected data was subjected to be analyzed by (IBM SPSS statistic version 21) Analysis of variance (ANOVA). The means were separated using Least Significance Differences (LSD).

Table 2.1 : Accession number and passport

Accession passport	Accession No.	Accession passport	Accession No.
HSD 4596	1	HSD 13711	31
HSD 5464	2	HSD 11464	32
HSD 3623	3	HSD 13258	33
HSD 6697	4	HSD 13809	34
HSD 3628	5	HSD 13639	35
HSD 5453	6	HSD 11425	36
HSD 4607	7	HSD 5451	37
HSD 4603	8	HSD 6705	38
HSD 4623	9	HSD 6728	39
HSD 6679	10	HSD 13682	40
HSD 13194	11	HSD 5467	41
HSD 4626	12	HSD 11463	42
HSD 13291	13	HSD 13280	43
HSD 5472	14	HSD 13204	44
HSD 3626	15	HSD 11401	45
HSD 6770	16	HSD 13305	46
HSD 13264	17	HSD 3619	47
HSD 3620	18	HSD 4593	48
HSD 13304	19	HSD 4599	49
HSD 13697	20	HSD 6696	50
HSD 5899	21	HSD 5462	51
HSD 5457	22	HSD 3621	52
HSD 13214	23	HSD 5448	53
HSD 5471	24	HSD 5452	54
HSD 4930	25	HSD 13202	55
HSD 5455	26	HSD 5463	56
HSD 13203	27	HSD 4928	57
HSD 5888	28	HSD 5479	58
HSD 13208	29	HSD 5461	59
HSD 13251	30	HSD 5901	60

2.3 Result and discussion

2.3.1 Plant height (cm)

As shown in table (2.2) and figure (2.1) significant differences ($p \geq 0.05$) in plant height between sesame genome types. A significant variation in plant height among different sesame genotypes has been reported by (Negasa 2014; Ngala *et al.* 2013). The results reported by (El Naim *et al.* 2012) obtained for sesame, also agreed with this result where they reported that plant height varied among different sesame genotypes. On the other hand, the result, by Naseri (2012) reported statistically non-significant differences on plant height of sesame types due to effects of variety. The different between varieties in the plant length was differenced to the origin, of the variety as the varieties were collected from different regions in Sudan for examples (north kordfan, south kordfan, west Darfour, Blue Nile and gadarf) source Agricultural Plant Genetic Resources Conservation and Research Centre) The plant height range between 95 to 203 Cm with the highest plant in south kordfan and gadarf. this result agrees with (Bennett and Condé, 2003). He said that *S. indicum* is an erect annual plant that can grow to a height of 1.0 to 2.0 meters depending on the cultivar and the growing conditions. However, varieties that are 1.0 to 1.4 m high are more common (Ashri and Singh, 2006).

2.3.2 Numbers of capsules per plant

Analysis of variance was showed that significant differences ($P \geq 0.05$) in number of capsules per plant between the sesame genotypes (table 2.4, fig. 2.3). (Abdalla Jumalla 2017) the observed were non-significant differences in number of capsule per plant in sesame genotypes tested. The possibility for variation among genotypes regarding that capsule number have been

attributed to the adaptation to the length of day and directly related to the flower number per plant, which can be seriously affected by the climatic conditions (Sogut 2008). The economic part of the plant is directly related to number of branches and the total number of capsules per plant.

Table 2.2: plant height (cm)

Sample number	Variety	Plant height	Sample number	Variety	Plant height
1	HSD 4596	95.0000	31	HSD13711	156.6667
2	HSD 5464	160.0000	32	HSD11464	138.3333
3	HSD 3623	130.0000	33	HSD13258	151.6667
4	HSD 6697	163.3333	34	HSD13809	185.0000
5	HSD 3628	143.3333	35	HSD13639	115.0000
6	HSD 5453	123.3333	36	HSD11425	126.6667
7	HSD 4607	158.3333	37	HSD 5451	177.5000
8	HSD 4603	203.3333	38	HSD 6705	163.3333
9	HSD 4623	173.3333	39	HSD 6728	136.6667
10	HSD 6679	135.0000	40	HSD13682	180.0000
11	HSD13194	111.6667	41	HSD 5467	170.0000
12	HSD 4626	173.3333	42	HSD11463	126.6667
13	HSD13291	203.3333	43	HSD13280	146.6667
14	HSD 5472	113.3333	44	HSD13204	181.6667
15	HSD 3626	123.3333	45	HSD11401	180.0000
16	HSD 6770	170.0000	46	HSD13305	196.6667
17	HSD13264	185.0000	47	HSD 3619	121.6667
18	HSD 3620	133.3333	48	HSD 4593	140.0000
19	HSD13304	183.3333	49	HSD 4599	166.6667
20	HSD 13697	168.3333	50	HSD 6696	160.0000
21	HSD 5899	180.0000	51	HSD 5462	160.0000
22	HSD 5457	134.0000	52	HSD 3621	128.3333
23	HSD 13214	198.3333	53	HSD 5448	176.6667
24	HSD 5471	133.3333	54	HSD 5452	183.3333
25	HSD 4930	140.0000	55	HSD 13202	185.0000
26	HSD 5455	176.6667	56	HSD 5463	135.6667
27	HSD 13203	180.0000	57	HSD 4928	141.6667
28	HSD 5888	195.0000	58	HSD 5479	133.3333
29	HSD 13208	106.6667	59	HSD 5461	172.0000
30	HSD 13251	123.3333	60	HSD 5901	160.0000

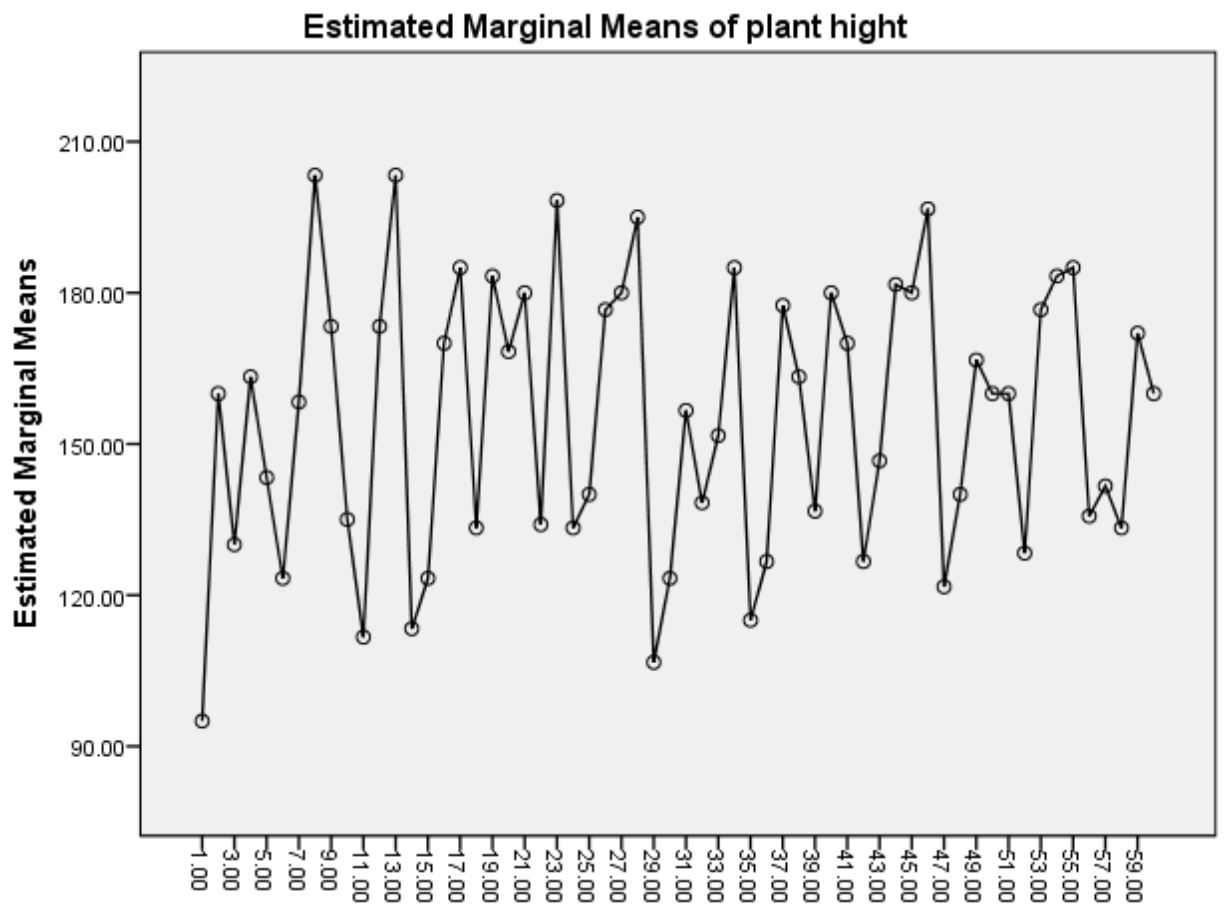


Figure 2.1 Sample numbers vs plant height (cm)

Table 2.3 : number of branches per plant

Sample number	Variety	Number of branches	Sample number	Variety	Number of branches
1	HSD 4596	2.3333	31	HSD13711	5.0000
2	HSD 5464	3.0000	32	HSD11464	1.6667
3	HSD 3623	5.0000	33	HSD13258	4.3333
4	HSD 6697	5.6667	34	HSD13809	4.0000
5	HSD 3628	6.6667	35	HSD13639	5.5000
6	HSD 5453	3.3333	36	HSD11425	7.3333
7	HSD 4607	5.0000	37	HSD 5451	2.7500
8	HSD 4603	3.0000	38	HSD 6705	6.3333
9	HSD 4623	4.3333	39	HSD 6728	3.3333
10	HSD 6679	3.3333	40	HSD13682	5.0000
11	HSD13194	4.0000	41	HSD 5467	3.3333
12	HSD 4626	2.6667	42	HSD11463	5.3333
13	HSD13291	4.3333	43	HSD13280	3.6667
14	HSD 5472	4.3333	44	HSD13204	3.6667
15	HSD 3626	4.6667	45	HSD11401	8.6667
16	HSD 6770	5.0000	46	HSD13305	5.3333
17	HSD13264	5.6667	47	HSD 3619	6.6667
18	HSD 3620	5.0000	48	HSD 4593	2.6667
19	HSD13304	3.3333	49	HSD 4599	3.3333
20	HSD 13697	4.3333	50	HSD 6696	5.0000
21	HSD 5899	4.6667	51	HSD 5462	2.3333
22	HSD 5457	3.6667	52	HSD 3621	4.0000
23	HSD 13214	3.6667	53	HSD 5448	1.6667
24	HSD 5471	5.3333	54	HSD 5452	4.3333
25	HSD 4930	2.6667	55	HSD 13202	6.3333
26	HSD 5455	4.3333	56	HSD 5463	4.3333
27	HSD 13203	5.6667	57	HSD 4928	3.3333
28	HSD 5888	4.6667	58	HSD 5479	2.3333
29	HSD 13208	6.6667	59	HSD 5461	2.6667
30	HSD 13251	3.3333	60	HSD 5901	3.6667

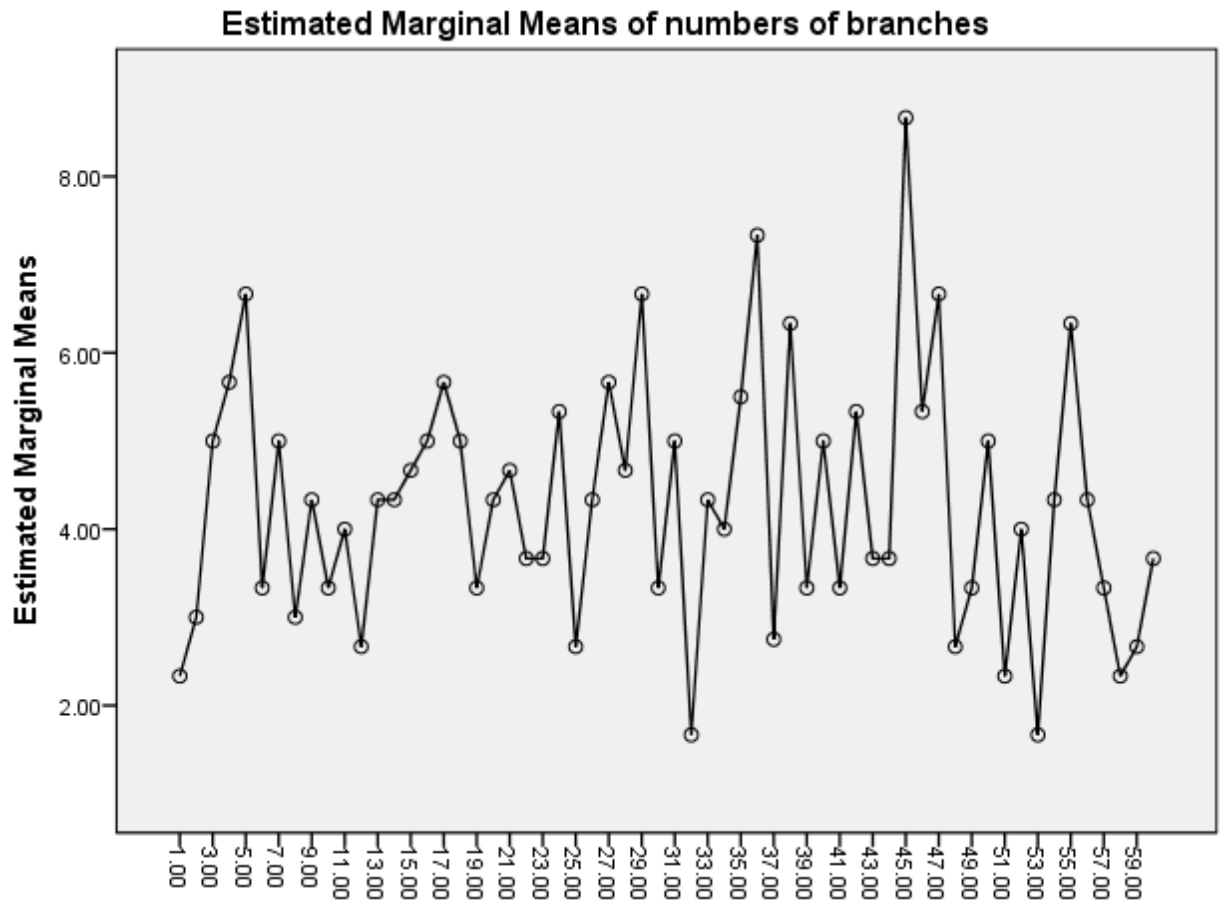


Figure 2.2 number of branches per plant

Table 2.4: number of capsules per plant

Sample number	Variety	Number of capsules	Sample number	Variety	Number of capsules
1	HSD 4596	32.0000	31	HSD13711	35.0000
2	HSD 5464	48.6667	32	HSD11464	18.6667
3	HSD 3623	42.0000	33	HSD13258	81.6667
4	HSD 6697	105.6667	34	HSD13809	110.6667
5	HSD 3628	116.0000	35	HSD13639	64.0000
6	HSD 5453	33.3333	36	HSD11425	79.3333
7	HSD 4607	49.3333	37	HSD 5451	26.7500
8	HSD 4603	69.3333	38	HSD 6705	99.3333
9	HSD 4623	139.0000	39	HSD 6728	22.0000
10	HSD 6679	77.6667	40	HSD13682	34.0000
11	HSD 13194	85.0000	41	HSD 5467	59.6667
12	HSD 4626	56.0000	42	HSD11463	63.3333
13	HSD 13291	141.0000	43	HSD13280	35.6667
14	HSD 5472	50.3333	44	HSD13204	54.0000
15	HSD 3626	23.3333	45	HSD11401	109.0000
16	HSD 6770	44.0000	46	HSD13305	78.6667
17	HSD13264	192.6667	47	HSD 3619	85.3333
18	HSD 3620	97.0000	48	HSD 4593	36.3333
19	HSD13304	82.3333	49	HSD 4599	43.3333
20	HSD13697	39.0000	50	HSD 6696	88.3333
21	HSD 5899	22.0000	51	HSD 5462	37.6667
22	HSD 5457	43.3333	52	HSD 3621	116.6667
23	HSD 13214	54.6667	53	HSD 5448	37.0000
24	HSD 5471	61.6667	54	HSD 5452	33.3333
25	HSD 4930	32.3333	55	HSD13202	103.3333
26	HSD 5455	66.0000	56	HSD 5463	30.0000
27	HSD 13203	70.0000	57	HSD 4928	63.6667
28	HSD 5888	120.6667	58	HSD 5479	29.0000
29	HSD 13208	35.3333	59	HSD 5461	52.6667
30	HSD 13251	49.0000	60	HSD 5901	91.3333

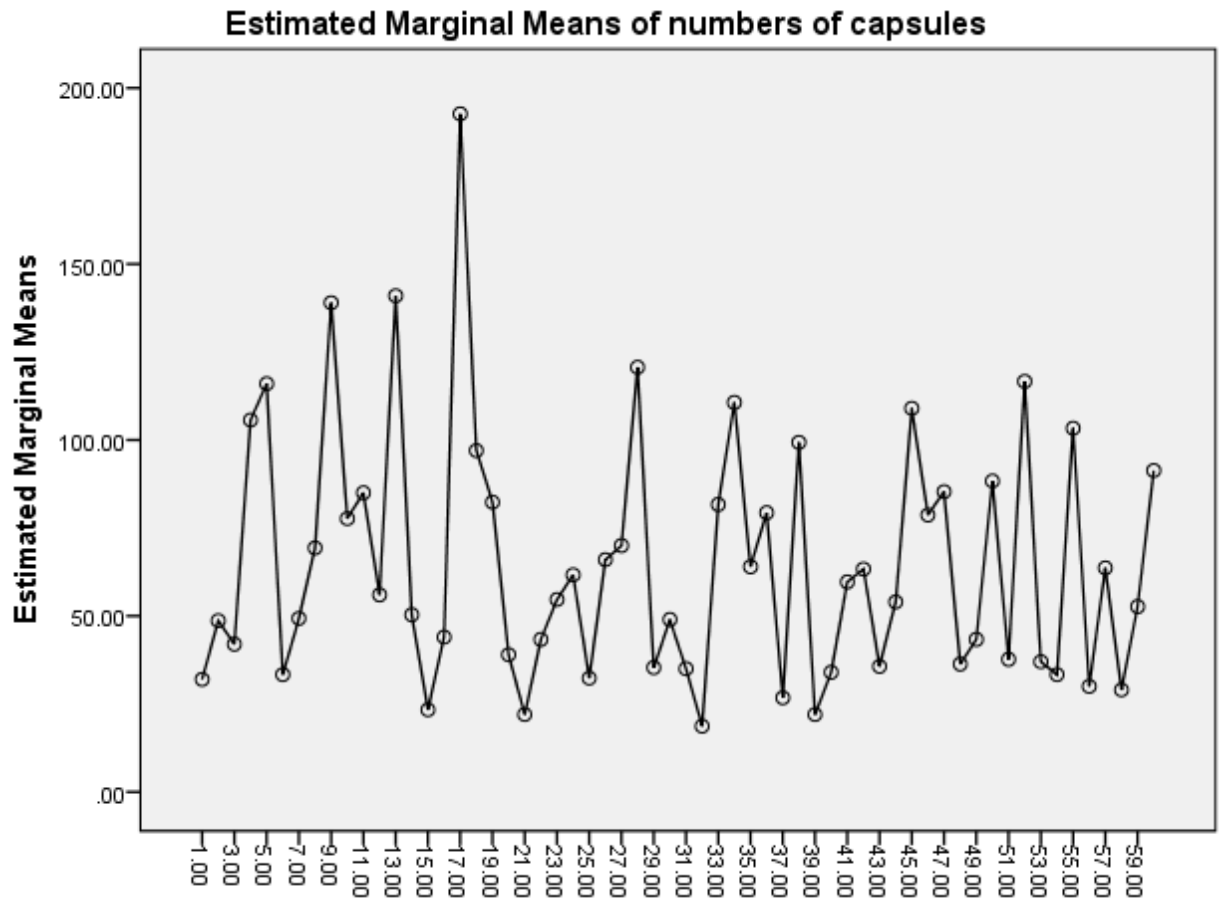


Figure 2.3 number of capsules per plant

2.3.3 Regression model between the variables

Regression model between the independent variables (No. Of branches, plant height) and the dependent variable (No. of capsules)

Table 2.5: Regression model between the variables

Parameters	Parameter value	Calculated t-test value	Probability value
Constant	- 47.6	-1.847	0.070
no. of branches	11.33	4.108	0.000
plant height	.412	2.838	0.000

Through the above table (2.5) fig. (2.4) and fig. (2.5), we notice that the probability value of the t-test for the variable no. of branches is equal to 0.000, which means that this variable significantly affects productivity. We also note that the probability value of the t-test for the variable plant height is equal to 0.000, which means that this variable significantly affects productivity. The estimated regression model between the independent variables (number of branches and plant height) and the variable is shown in the following equation

$$y = -47.6 + 11.33\text{No. of branches} + .412\text{plant hight}$$

Interpretation of the value of the number of branches: If the independent variable increases by one unit, then productivity will increase by 11.33. Interpretation of the plant height coefficient value: If the independent variable increases by one unit, then productivity will increase by 0.412.

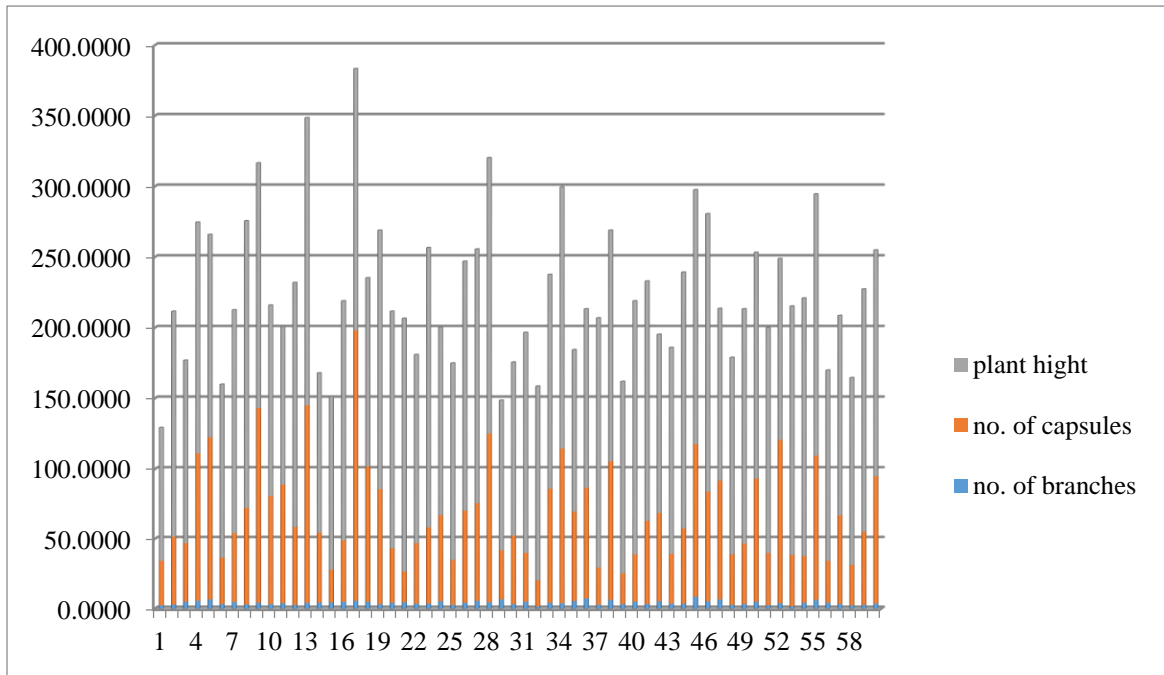


Figure 2.4 compression between plant height , no. of branches and no. of capsules

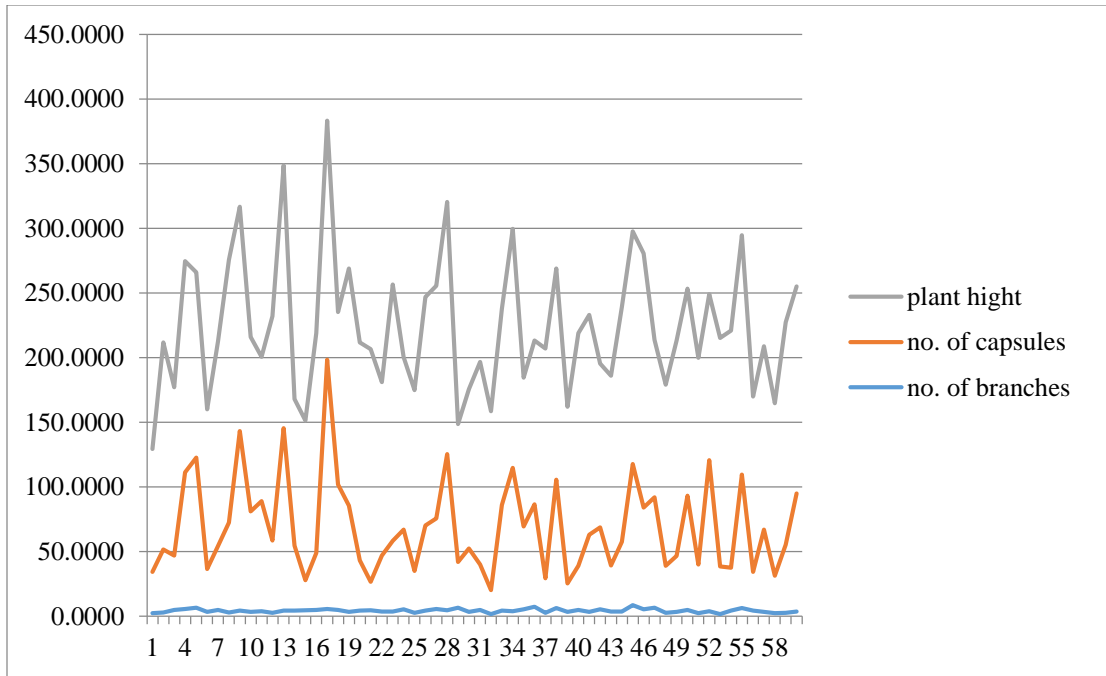


Figure 2.5 compression between plant height , no. of branches and no. of capsules

Chapter three

The Effect of Abiotic Stress on Germination and Growth of Sesame Genotypes

Chapter three

3.1 Literature review (Stresses)

3.1.1 Abiotic and biotic stress

In the past decades, tremendous progress has been made in understanding the mechanisms underlying plant resistance/tolerance to individual biotic and/or a biotic stress. Moreover, plant responses to various stresses and their positive or negative impacts on plant growth have been comprehensively studied (Poltronieri, *et al.* 2011).

Plant physiology is significantly affected by a biotic/climatic stresses. It is well known that climate change and environmental extremes induce and enhance the impact of abiotic stresses (particularly drought and salinity) on plant fitness and performance (Kaushal and Wani 2016). The physiological quality of seeds is an aspect of great relevance for the establishment of a crop. Physical, physiological, genetic and sanitary aspects related to seed quality are considered as attributes that determine the value of the sowing and, consequently, the establishment of the crop, a basic factor to ensure a successful production (Barrozo *et al.* 2012).

Environmental conditions and techniques adopted during the production of seeds may influence their physiological quality, and their vigor may or may not be affected more intensely (Marcos Filho 2013), especially if subjected to stress conditions. The effect of saline stress during the seed production phase may cause losses in transport and accumulation of reserves, resulting in low quality seeds and vigor. Thus, the quality of the seed will reflect on the stand of plants, thus affecting the performance of the plant, both from the nutritional

point of view as in quality (Neta *et al.* 2016). Environmental stresses specially drought, play role in decreasing plant growth, particularly during germination in dry and semi dry area (Hossein and Keshavarzi 2012).

climatic stresses are currently the major topics of interest for agriculture and plant-based biotechnologies resistance to Plant growth, development and productivity. Both biotic (e.g., phytopathogens) and a biotic stress (e.g., drought, salinity, flood, storm, and extreme temperatures) cause enormous losses in agricultural production (Fraire, *et al.* 2013).

The osmotic effect caused by salts reduces the capacity of water absorption by plants, in addition to the effects of some specific ions, particularly Na⁺ and Cl⁻, which lead to functional disorders and injuries, especially in the leaves, culminating in modifications in physiological and metabolic processes, which may affect the crop yield quality and final quality of the product (Nobre *et al.* 2013).

Salt stress adversely affects the growth of plants during all development stages (DEOG, *et al.* 2006). Sesame has moderate salt tolerance, but will not grow under flooded conditions. Selection and growing of salt tolerant food and forage crops could increase productivity and production of crops in marginalized salt affected land (Mamo *et al.* 2021).

As the most important a biotic factor for limiting growth, Drought stress adversely affects crop growth and production. Droughts affect photosynthesis through stomatal closure and not reach carbon dioxide to chloroplasts and decreasing in water potential of cells. The average precipitation of 240 ml has about one-third of the annual rainfall (700 mm), its climate is arid and Semi-

arid. According to the average rate of population growth, it is estimated that the need for water increases to 2-fold every 35 years (Kumar, *et al.* 2003).

3.1.2 Abiotic stress (Drought and salinity)

A biotic stress such as drought and salinity are major environmental factors that influence crop productivity worldwide. These adverse conditions induce osmotic stresses in plant cells by decreasing water availability, thus leading to loss of cell turgor and the accumulation of reactive oxygen species (ROS) that are detrimental to plant growth and development. To survive such harsh environmental conditions, plants must initiate intracellular and physiological signaling networks to rapidly respond and efficiently neutralize these stresses. Inefficient scavenging of ROS would lead to increased levels of cell death, thus inhibiting plant growth and reducing crop productivity (Nxele *et al.* 2017).

Drought and salinity are two major abiotic stresses due to their wide occurrence and high magnitude of their impact (Bartels and Sunkar 2005) Severe drought and high salinity could promote land desertification and salinization, processes which are rapidly increasing on a global scale. More than 10% of arable land has become desertified or salinized, and average yields of major crops have been reduced by more than 50% (Wu *et al.* 2011).

Drought and salinity are among the most important environmental factors that hampered agricultural productivity worldwide. Both stresses can induce several morphological, physiological, biochemical and metabolic alterations through various mechanisms, eventually influencing plant growth, development, and productivity. The responses of plants to these stress conditions are highly complex and depend on other factors, such as the species

and genotype, plant age and size, the rate of progression as well as the intensity and duration of the stresses. These factors have a strong effect on plant response and define whether mitigation processes related to acclimation will occur or not (Ma, *et al.* 2020).

Early responses to drought and salinity are very similar since both induce water stress that leads to a slowdown in growth, a decrease in stomatal aperture, and a nutrient deficiency (such as K⁺ and Ca²⁺). However, during long term exposure to salt, besides dehydration, plants experience ionic stress, which leads to leaf senescence and photosynthesis impairment (that in turn exerts an additional negative effect on growth. Photosynthesis is one of the main processes affected by drought and salinity (Chaves, *et al.* 2009).

Drought and salinity are major environmental factors that influence crop productivity worldwide. These abiotic stress conditions result in biochemical changes such as the accumulation of ROS that are byproducts of cellular metabolism (Pan, *et al.* 2006).

Salinity and drought stresses are physiologically related because both induce osmotic stress and most of the metabolic responses of stressed plants are similar (Kumar and Sharma 2011)

Drought, salinity, extreme temperature, and oxidative stress are interconnected abiotic stresses damaging plant cell through morphological, physiological, biochemical, and molecular changes adversely affecting growth and productivity (Jewell *et al.* 2010).

(*Sesamum indicum* L.) moderately tolerant to drought and salinity is increasingly cultivated. It is an alternative to fight poverty in rural areas and allows the revaluation of salty land. (Dangue *et al.* 2022). Sesame has great

potential as an industrial crop but its production is challenged by drought and salt stresses. To unravel the genetic variants leading to salinity and drought tolerances at the germination stage, genome-wide association studies of stress tolerance indexes related to NaCl- salt and polyethylene glycol-drought induced stresses were performed with a diversity panel of 490 sesame accessions. An extensive variation was observed for drought and salt responses in the population and most of the accessions were moderately tolerant to both stresses.

3.1.2.1 Drought stress

Sesame is an important oilseed crop, there is very limited quantitative information about the sesame responses to drought stress during seed germination and seedling establishment. Measures of drought responses based on germination indices and seedling traits under controlled and stress conditions have been previously used by researchers to quantify the drought tolerance in different plant species (Patanè, 2013). Recently, stress tolerance and drought susceptibility indices were reported to be the most useful indicators to measure the seed germination and seedling development responses to the drought (Grzesiak *et al.* 2019).

Drought is one of the most important abiotic stress that impairs sesame (*Sesamum indicum L.*) productivity mainly when it occurs at flowering stage. However up to now, very few studies have attempted to investigate the molecular responses of sesame to drought stress (Dossa, *et al.* 2017).

As the most important abiotic factor for limiting growth, Drought stress adversely affects crop growth and production. Droughts affect photosynthesis

through stomatal closure and not reach carbon dioxide to chloroplasts and decreasing in water potential of cells (Kumar, *et al.* 2001).

Drought stresses as the most important a biotic factor for limiting growth, adversely affects crop growth and production. (Farokhian, *et al.* 2021).

Drought is considered one of the abiotic constraint to agricultural crop production globally. Present study was conducted to assess the effects of different drought treatments (*viz.* Control, 10% PEG, and 20% PEG) on seed germination, germination indices, seedling traits, and drought tolerance indices of sesame. drought impact on seed germination and seedling traits could be quantified by using different indices which can further help to design drought adaptation and mitigation strategies. drought tolerance indices have great potential to simulate drought stress impacts on different crop traits thus they should be used in all kinds of stress related studies (Ahmed, *et al.* 2022).

Increased drought is one of the consequences of global climate change in regions, arid and semi-arid areas particular in. Sesame is adaptable to drought condition which could produce a proper amount of good quality oil. showed that accessions with higher index (STI) had more grain weight per capsule, higher capsules number per plant, and longer petiole in lower leaf that measured in normal condition (Abbasali, *et al.* 2017).

Drought tolerance consists of plant ability to growth under water deficit conditions. Understanding the response of plant to dry environments has great importance and also a fundamental part of producing stress tolerant crops. Increasing plant resistance to drought stress would be the most economical approach to improve agricultural productivity and reduce agricultural use of fresh water resources. Drought is greater challenge that decreases agricultural

production substantially all over world. It induces several common physiological responses in plants, such as water relation and photosynthetic ability (Hota, *et al.* 2019).

Turk *et al.* (2004) found that one of the reasons that can reduce or delay or even prevent germination is water stress. It also decreases germination rate and seedling growth rate. There were some studies that using local sesame from Nigeria which found that low level of drought stress hadn't any significant effect on germination, by increasing levels of drought germination and seedling growth reduced, on the other hand, drought stress level has negative correlation with germination and seedling growth (Mensah, *et al.* 2006).

In sesame, several studies have reported that many traits including germination rate, seedling growth, shoot length, root length and yield related traits could be affect by drought stress (Sun, *et al.* 2010).

Sesame is a resilient crop with a strong adaptation to drought-prone environments. As compared to other major food crops, sesame better survives drought (Islam, *et al.*, 2016). As a consequence, sesame seed yields are generally low (300–400 kg/ha) in most of the arid and semi-arid areas (Islam, *et al.* 2016).

Drought significantly affects the germination indices and seedling characteristics and severe drought at these stages even leads to total crop failure (Petrović *et al.* 2016).

Compared to other crops, sesame has better drought tolerance; however, it remains particularly sensitive to drought occurring during germination and seedling stages (Seyni Boureima, *et al.* 2011). Improvement in sesame

(*Sesamum indicum* L.) drought tolerance at seedling stage is important for yield stability. Genetic approaches combining with conventional breeding is the most effective way to develop drought-tolerant cultivars. So far, only a few studies have been reported to reveal gene quantitative trait loci (QTL) controlling drought tolerance in sesame (Liang, *et al.* 2020).

In order to evaluate traits related to drought stress in eight sesames (*Sesamum indicum* L.) genotypes. Unlike most of the important food crops, sesame can survive drought but severe and repeated drought episodes, especially occurring during the reproductive stage, significantly curtail the productivity of this high oil crop. This study also revealed that pyramiding the favorable alleles observed at the peak loci is of high potential for enhancing drought tolerance in sesame (Dossa, *et al.* 2019).

Drought stress can occur at any growth stage and can affect crop productivity, which can result in large yield losses all over the world. In this respect, understanding the genetic architecture of agronomic traits under drought stress is essential for increasing crop yield potential and harvest. Barley is considered the most a biotic stress-tolerant cereal, particularly with respect to drought (Thabet, *et al.* 2020).

The effects of drought on germination of sesame seeds (*Sesamum indicum* L.) The growth and seed yield of sesame are adversely affected by continuous flooding and severe drought (Mensah, *et al.* 2006).

Improvement in sesame drought tolerance at seedling stage is important for yield stability. Genetic approaches combining with conventional breeding is the most effective way to develop drought-tolerant cultivars Further, we have also presented the scope of conventional and speed breeding platforms in helping

to develop the drought-smart future crops. In short, we recommend incorporating several approaches, such as multi-omics, genome editing, speed breeding, and traditional mechanical strategies, to develop drought-smart cultivars to achieve the ‘zero hunger’ goal (Raza, *et al.* 2022).

Drought is the main constraint for crop growth worldwide. Selenium reportedly plays an important role in improving plant tolerance to drought stress. Drought stress was triggered 50 days after sowing, and selenium was sprayed 50, 55, and 60 days after sowing. This finding confirms that selenium can be applied to enhance sesame’s tolerance to drought stress (Thuc *et al.* 2021).

3.1.2.2 Salinity stress

Salinity is one of the most serious factors limiting crop production and productivity mainly in arid and semiarid regions (Fazeli, *et al.* 2012). Soil is considered to be saline when its electrical conductivity is 4 ds/m (equivalent to 40 mM) or more (FAO 1997). Plants use different mechanism to overcome effect of soil salinity.

At cellular level, plants cope up with salinity by osmotic adjustment involving vacuolar sequestration of ions and synthesis of osmo protectants in the cytoplasm (Garg, *et al.* 2002). At molecular level, plants synthesize stress proteins that may have diverse functions in regulating the effect of salinity. These may contribute to detoxification pathway in many forms such as part of scavenging enzyme, or help in the synthesis of antioxidants (Mittova, *et al.* 2002).

Salinity is one of the major environmental stresses affecting plant growth and development and results in severe agricultural losses. It is one of the most

serious factors limiting crop production and productivity mainly in arid and semiarid regions, It affects nutrient uptake and metabolic activities in the plants (Fazeli *et al.* 2012).

Salinity is a global problem, and salt affected areas are rapidly increasing due to low rainfall, poor irrigation systems, saline irrigation water and other environmental factors (Mishra and Tanna, 2017).

High salinity affects plants in different ways; namely water stress, ion toxicity, nutrient deficiency, oxidative stress, alteration of metabolic processes, membrane disorganization, and impaired cell division and expansion as a result ion toxicity leads to chlorosis and necrosis, mainly due to Na⁺ accumulation in cytoplasm, which affects many physiological processes in plants (Munns, 2002). The entry of both Na and + Cl into the cells causes severe ion imbalance, and excess - absorption may cause significant physiological disorders (Ketehouli, *et al.* 2019).

Salinity is a serious challenge affecting our food production today. While some plants can tolerate salts some cannot. To what extent salinity affects growth and yield of plants sesame needs to be investigated, Salinity is one of the most severe a biotic stress that limits crop production and productivity especially in arid and semiarid areas of the world. It causes morphological, physiological, biochemical and molecular changes and adversely affects plant growth and metabolism Salinity affects negatively germination of sesame, seedling growth of sesame and growth parameters (plant height number of leaves shoot fresh and dry weight) which decrease with increase in salinity level. However, crops respond and perform differently when exposed to salinity and some can be tolerant. The sesame crop is usually avoided in salt-affected areas because of the various effects of saline stress on plants. Besides

varying between species, salinity effects are known to vary for genotypes of the same species as well as plant development stages (Suassuna, *et al.* 2017). Therefore, selection and characterization of germplasm is needed to obtain salt tolerant crops (Muhammad, *et al.* 2018).

Sesame is considered an alternative crop for small and medium farmers in the Brazilian Northeast region. However, under the conditions of the northeastern semi-arid region, the scarcity of good quality water for irrigation may lead to a reduction in the quality of the final product (Nóbrega, *et al.* 2018)

3.2 Materials and Methods (Stress)

3.2.1 Experimental Materials

The experiment was conducted in central laboratory research of Shendi University, to study the stress effect (salinity and drought) on germination and morphological characterization of *Sesame indicium* seedlings. A Complete randomize book design (CRBD) in a 60×3 factorial scheme, with three replicates was adopted. The stander germination test was applied to study the effects of both salinity and drought stresses.

3.2.1.1 Sesame seed

The sixty accessions out of 100 accession from the previous study as demonstrated in chapter Two (2.2.2) was selected to conduct the stress experiment (drought and salinity) (table 3.1). Thirty seed from each of the sixty accessions selected were handily sorted and cleaned to use in the study.

3.2.1.2 Petri dishes and filter paper

Disposable petri dishes 90 mm were used, Whatman Filter paper 90 mm was placed in the petri dishes and divided into two equal zones. one of the zones

was divided into four smaller zones. Moreover, each zone of the small four zones was labeled based on the height of the germinated seeds (0.1-0.3 cm), (0.4-0.6 cm), (0.7-1 cm) and above 1 cm. figure 3.1 (A)

3.2.2 Excrement (1) drought stress

Three different concentrations of polyethylene glycol (PEG 6000) were prepared by dissolving a certain amount of the salt in one liter deionized water as following:

(PEG 6000), (0 bar = 0g/L. 6 bar = 189 g/L, 9 bar =222 g/L).

3.2.2.1 Excrement layout (60×3×3)

Thirty selected seed of each accession (3.2.1.1) were placed in the undivided zone of the filter paper in the petri dishes (3.2.1.2)

3.2.2.1.1 Treatment

The filter paper with the seed were sprayed by each of the PEG concentration under test (0, 6, 9) bar. all the petri dishes were left at room temperature (24-28°C) by exposing them to the dark 16 hours and the light for 8 hours.

3.2.2.1.2 Sampling

After 24 hours then the first reading of the growth rate were taken by measuring the height of seedlings by a ruler and transferred using a sterilized forcep to the first small zone (0.1- 0.3 cm). in the second reading after six hours transfer to one of the divided zones according to the length of the seedling. This was repeated two times each day (six hours between samples for five days).

The previous experiment was left at room temperature for five days. five seedlings were selected randomly from each petri dish. shoots and roots of the

selected seedling were measured using a ruler and the shoot –root ratio was calculated.

The total germination percentage (TG) and the normal(NS) and abnormal (AB) seedlings were measured.

The germinator software(Joosen *et al.* 2010) was used to calculated the germination speed parameters (AUC, T50 and T10).

3.2.3 Experiment (2) salinity stress

Three different concentrations of sodium chloride (NaCl) were prepared by dissolving a certain amount of the salt in one liter deionized water as following:

NaCl, (0 mM= 0 g/L, 60 mM = 3.5g/L and 80 mM = 4.7g/L).

Experiment 1 (drought stress) was repeated with sodium chloride concentrations (0, 60 ,80) Mm in state of PEG6000. The experiment follows the same layout, methods of appalling treatment, sampling and shoot and root measurement.

3.2.4 Data collection and analysis

Based on the recorded data, the stress tolerance (ST) index was estimated as the ratio of the number of germinated seeds using excel germinator software. The shoot and root length data collected from the above two experiments (3.2.2, 3.2.3) were Analyzed using IBM SPSS statistic version 21this analysis of variance (ANOVA) test $p \leq 0.05$ (re) .

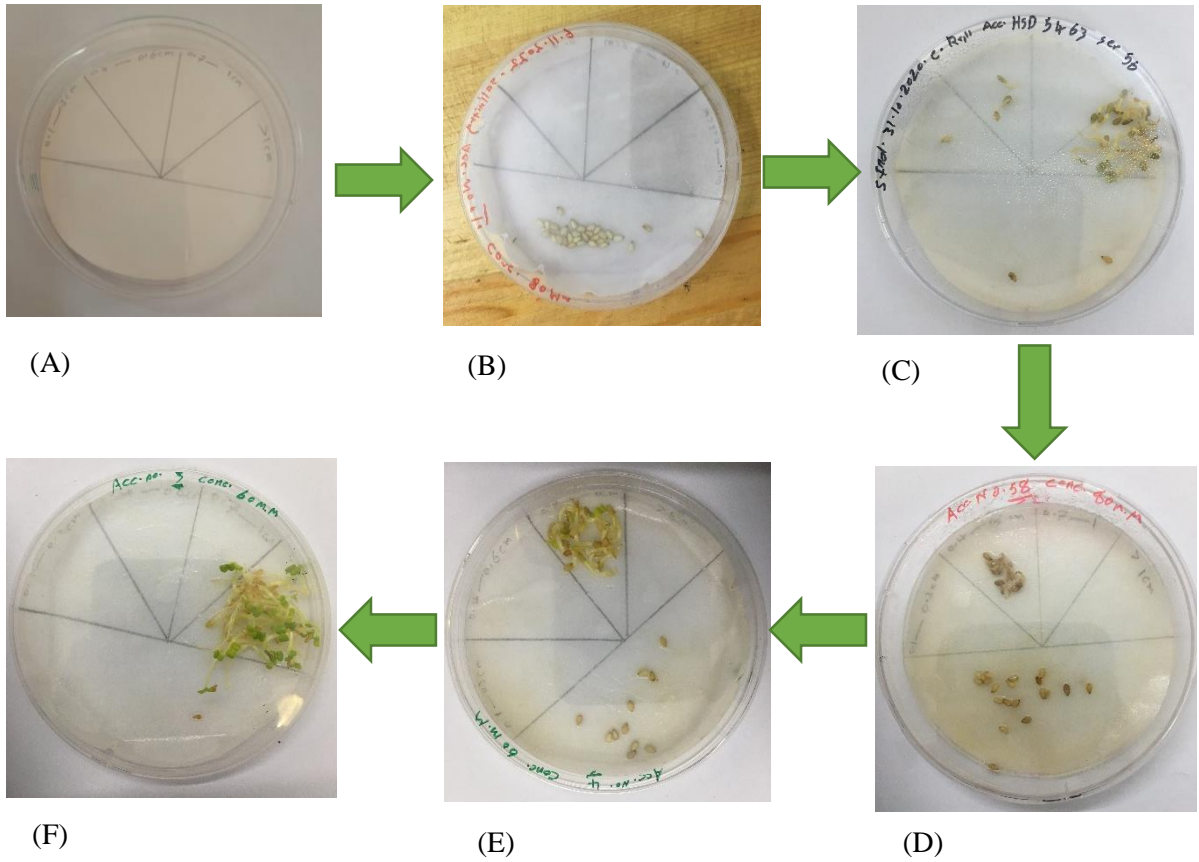


Figure 3.1 the process of seed treatment in abiotic stress (drought and salinity)

3.3 Result and discussion

3.3.1 Germination

3.3.1.1 Effect of drought stress on Sesame germination

Table 3.1 and figure 3.2 showed the effect of drought stress on germination sesame the results indicated significant ($P \leq 0.05$) differences in sesame germination compared to the control untreated seeds. From the results it was shown that increasing the concentration of polyethylene glycol zero to 6 and 9 bar decreased the percentage of germination percentage. The percentage of seed germination ranged between (40 - 47%) and (83 – 100%). Hossein and kesharvargi, 2012 reported a decrease in sesame germination due to increase in the drought stress. Likewise, grgiziate *et al* (2019) showed that stress tolerance and drought susceptibility indices were the most useful indicators to measure the seed germination and seedling development response to drought.

3.3.1.2 Effect of Salinity stress on Sesame germination

Significant differences ($P \leq 0.05$) in sesame seed germination in table (3.2) figure 3.3 were observed when the concentration of NaCl salinity stress was increased from zero to 60 and 80 mM in most of the sesame varieties by different percentage. Seed germination was found to be in the range of (86 to 100%) in the control untreated seed where as these range 52 – 97 % at 60 mM and 32 – 90 % at 80 mM conc. Indicating the effect of salinity on seed germination percentage. Many researchers found that increasing the concentration of sodium chloride decreased seed germination (Adamu and Abubaker, 2018 and Ahmed *et al* 2022). As well (Mensah. *et al*. 2006) reported a significant reduction in seed germination as result of addition of polyethylene glycol, glucose and sodium chloride.

Most of the previous studies had obtained similar result with this study, (Muhammad *et al.* 2018) Found that the effect of different level of sodium chloride salt on germination and seedling growth. In addition, Ahmed *et al.* (2022) said that higher PEG concentration of PEG as compared to control, the drought treatments decreased the values for germination percentage. As well as,(Mensah *et al.* 2006) reported that significant reduction in the percentage of the germination, the ability of the three chemicals to reduce germination in sesame was of the order: polyethylene glycol, glucose and sodium chloride.

Table 3.1: Effect of drought stress Polyethylene glycol (bar) on Sesame germination

Sample number	Accession No.	Germination %		
		D0	D6	D9
1	HSD 4596	97.78 ±2.222	92.22±2.222	30.00±20.000
2	HSD 5464	98.89±1.111	94.44±2.222	97.78±1.111
3	HSD 3623	96.67± 1.925	87.78±4.444	100.00±0.000
4	HSD 6697	96.67± 3.333	86.67±7.698	47.78±14.444
5	HSD 3628	95.56± 4.444	78.89±21.111	71.11±5.556
6	HSD 5453	91.11± 8.889	96.67±3.333	53.33±20.000
7	HSD 4607	100.00± 0.000	88.89±11.111	95.56±2.222
8	HSD 4603	88.89± 4.444	92.22±1.111	84.44±1.111
9	HSD 4623	97.78± 2.222	93.33±6.667	93.33±0.000
10	HSD 6679	96.67± 1.925	98.89±1.111	64.44±17.778
11	HSD13194	96.67± 1.925	85.561±1.111	95.56±2.222
12	HSD 4626	100.00± 0.000	58.89±18.889	91.11±5.556
13	HSD13291	97.78± 2.222	77.78±18.889	97.78±2.222
14	HSD 5472	94.44± 5.556	70.00±0.000	71.11±7.778
15	HSD 3626	100.00± 0.000	60.00±13.333	84.44±2.222
16	HSD 6770	90.00± 1.925	60.00±3.333	44.44±14.699
17	HSD13264	100.00± 0.000	97.78±2.222	83.33±6.667
18	HSD 3620	47.78± 2.940	40.00±3.333	43.33±1.925
19	HSD13304	97.78± 2.222	85.56±14.444	84.44±7.778
20	HSD 13697	98.89± 1.111	68.89±31.111	100.00±0.000
21	HSD 5899	96.67±1.925	88.89±8.012	95.56±2.222
22	HSD 5457	80.00± 6.939	54.44±5.556	45.56±2.222
23	HSD 13214	90.00± 6.939	72.22±27.778	90.00±0.000
24	HSD 5471	96.67± 1.925	94.44±5.556	80.00±13.333
25	HSD 4930	94.44± 5.556	81.11±8.889	68.89±15.556
26	HSD 5455	92.22± 2.940	88.89±6.186	87.78±2.940
27	HSD 13203	97.78± 2.222	90.00±10.000	86.67±6.667
28	HSD 5888	94.44± 5.556	100.00±0.000	67.78±1.111
29	HSD 13208	97.78± 1.111	82.22±17.778	95.56±2.222
30	HSD 13251	98.89± 1.111	93.33±3.333	97.78±1.111

Continue

Sample number	Accession No.	Germination %		
		D0	D6	D9
31	HSD13711	97.78± 2.222	84.44±1.111	84.44±1.111
32	HSD11464	91.11± 4.444	68.89±4.444	50.00±10.000
33	HSD13258	100.00± 0.000	98.89±1.111	85.56±1.111
34	HSD13809	90.00± 6.939	81.11±4.006	75.56±7.778
35	HSD13639	98.89± 1.111	100.00±0.000	42.22±21.111
36	HSD11425	96.67± 3.333	94.44±5.556	44.44±7.778
37	HSD 5451	100.00± 0.000	78.89±11.111	67.78±5.556
38	HSD 6705	97.78± 2.222	80.00±10.000	95.56±1.111
39	HSD 6728	93.33± 3.333	93.33±3.333	90.00±3.333
40	HSD13682	94.44± 4.006	85.56±7.286	85.56±1.111
41	HSD 5467	87.78± 6.186	82.22±17.778	60.00±16.667
42	HSD11463	100.00± 0.000	52.22±12.222	94.44±5.556
43	HSD13280	90.00± 6.939	91.11±8.889	83.33±6.667
44	HSD13204	97.78± 2.222	93.33±3.333	96.67±1.925
45	HSD11401	100.00± 0.000	92.22±7.778	66.67±10.000
46	HSD13305	95.56± 2.222	57.78±1.111	54.44±2.940
47	HSD 3619	98.89± 1.111	97.78±2.222	48.89±25.556
48	HSD 4593	98.89± 1.111	76.67±6.667	97.78±2.222
49	HSD 4599	100.00± 0.000	93.33±6.667	84.44±7.778
50	HSD 6696	95.56± 1.111	88.89±2.940	77.78±7.778
51	HSD 5462	98.89± 1.111	91.11±8.889	53.33±16.667
52	HSD 3621	96.67± 1.925	87.78±4.843	88.89±1.111
53	HSD 5448	92.22± 4.006	75.56±4.006	86.67±3.333
54	HSD 5452	96.67± 1.925	68.89±7.286	90.00±3.333
55	HSD 13202	98.89± 1.111	67.78±22.222	98.89±1.111
56	HSD 5463	92.22± 6.186	53.33±6.667	64.44±8.889
57	HSD 4928	96.67± 1.925	47.78±2.222	74.44±1.111
58	HSD 5479	86.67± 3.849	66.67±20.092	68.89±11.111
59	HSD 5461	98.89± 1.111	98.89±1.111	93.33±3.849
60	HSD 5901	88.89± 5.556	66.67±13.472	48.89±4.444

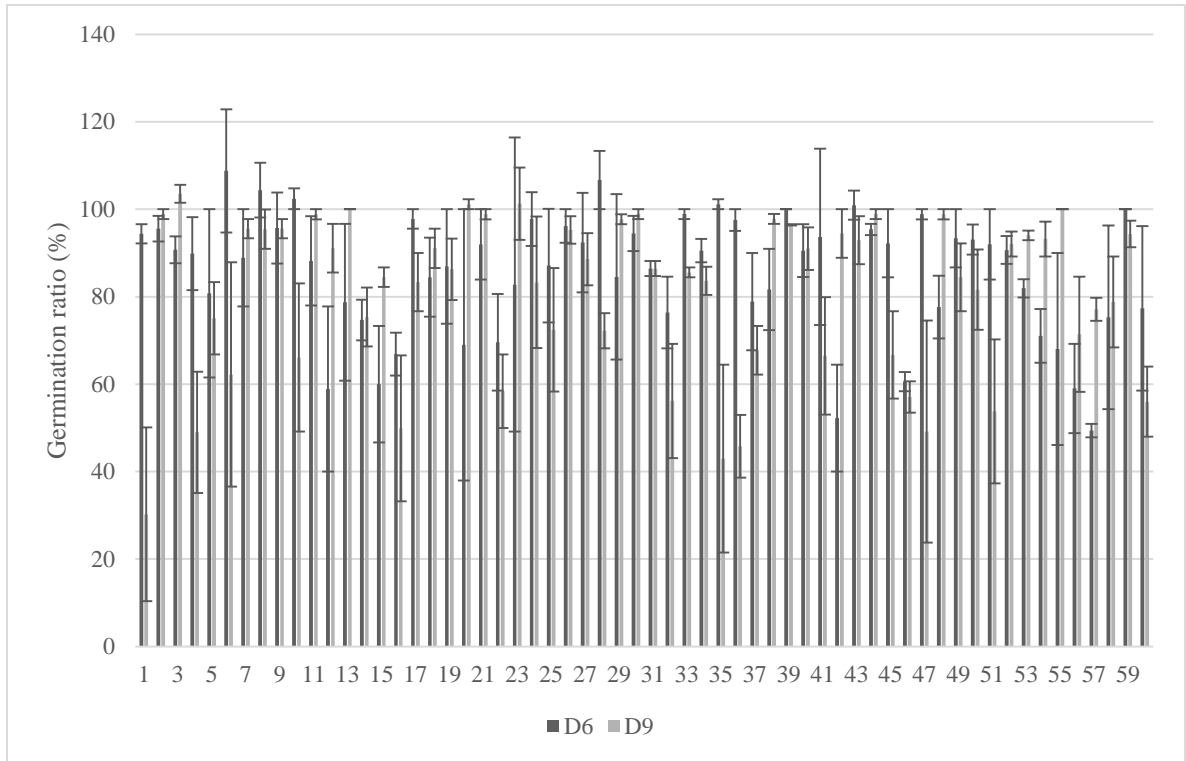


Figure 3.2 Effect of drought stress Polyethylene glycol (bar) on Sesame germination

Table 3.2: Effect of salinity stress Sodium chloride concentration(mM) on Sesame germination

Sample number	Accession No.	Germination %		
		S 0	S 60	S 80
1	HSD 4596	97.78 ±2.222	91.11±2.222	81.11±6.186
2	HSD 5464	98.89±1.111	66.67±5.092	82.22±2.940
3	HSD 3623	96.67± 1.925	94.44±1.111	74.44±5.556
4	HSD 6697	96.67± 3.333	57.78±2.940	32.22±2.940
5	HSD 3628	95.56± 4.444	85.56±4.843	63.33±1.925
6	HSD 5453	91.11± 8.889	78.89±7.286	63.33±3.333
7	HSD 4607	100.00± 0.000	88.89±2.222	76.67±5.774
8	HSD 4603	88.89± 4.444	87.78±5.556	74.44±6.186
9	HSD 4623	97.78± 2.222	85.56±2.940	66.67±3.333
10	HSD 6679	96.67± 1.925	91.11±2.222	58.89±11.277
11	HSD13194	96.67± 1.925	95.56±2.222	65.56±4.843
12	HSD 4626	100.00± 0.000	98.89±1.111	71.11±6.759
13	HSD13291	97.78± 2.222	90.00±1.925	88.89±6.186
14	HSD 5472	94.44± 5.556	77.78±2.222	26.67±5.092
15	HSD 3626	100.00± 0.000	68.89±15.556	57.78±16.140
16	HSD 6770	90.00± 1.925	3.33±0.000	22.22±9.876
17	HSD13264	100.00± 0.000	80.00±5.092	75.56±1.111
18	HSD 3620	47.78± 2.940	32.22±4.843	20.00±1.925
19	HSD13304	97.78± 2.222	90.00±5.092	72.22±4.006
20	HSD 13697	98.89± 1.111	88.89±4.444	73.33±5.092
21	HSD 5899	96.67±1.925	90.00±1.925	46.67±13.472
22	HSD 5457	80.00± 6.939	36.67±1.925	35.56±2.940
23	HSD 13214	90.00± 6.939	82.22±2.940	53.33±3.849
24	HSD 5471	96.67± 1.925	85.56±7.286	65.56±4.006
25	HSD 4930	94.44± 5.556	73.33±1.925	56.67±8.389
26	HSD 5455	92.22± 2.940	92.22±2.940	68.89±1.111
27	HSD 13203	97.78± 2.222	97.78±2.222	83.33±8.389
28	HSD 5888	94.44± 5.556	91.11±2.940	64.44±1.111
29	HSD 13208	97.78± 1.111	96.67±0.000	82.22±2.940
30	HSD 13251	98.89± 1.111	96.67±3.333	86.67±0.000

Continue

Sample number	Accession No.	Germination %		
		S 0	S 60	S 80
31	HSD13711	97.78± 2.222	93.33±1.925	66.67±1.925
32	HSD11464	91.11± 4.444	36.67±8.819	23.33±11.706
33	HSD13258	100.00± 0.000	97.78±2.222	57.78±7.778
34	HSD13809	90.00± 6.939	88.89±2.940	80.00±6.939
35	HSD13639	98.89± 1.111	87.78±4.006	81.11±5.556
36	HSD11425	96.67± 3.333	86.67±5.092	70.00±13.878
37	HSD 5451	100.00± 0.000	82.22±1.111	57.78±2.940
38	HSD 6705	97.78± 2.222	71.11±2.940	67.78±8.012
39	HSD 6728	93.33± 3.333	55.56±14.186	30.00±8.389
40	HSD13682	94.44± 4.006	92.22±4.444	77.78±2.940
41	HSD 5467	87.78± 6.186	57.78±6.186	42.22±11.277
42	HSD11463	100.00± 0.000	85.56±1.111	80.00±7.698
43	HSD13280	90.00± 6.939	95.56±2.940	78.89±1.111
44	HSD13204	97.78± 2.222	86.67±0.000	82.22±7.286
45	HSD11401	100.00± 0.000	94.44±2.222	85.56±4.444
46	HSD13305	95.56± 2.222	88.89±4.444	86.67±3.849
47	HSD 3619	98.89± 1.111	95.56±1.111	91.11±1.111
48	HSD 4593	98.89± 1.111	76.67±6.939	74.44±4.444
49	HSD 4599	100.00± 0.000	88.89±1.111	94.44±4.006
50	HSD 6696	95.56± 1.111	52.22±4.843	53.33±6.667
51	HSD 5462	98.89± 1.111	75.56±5.556	50.00±18.359
52	HSD 3621	96.67± 1.925	84.44±1.111	83.33±5.774
53	HSD 5448	92.22± 4.006	70.00±5.092	66.67±6.939
54	HSD 5452	96.67± 1.925	86.67±13.333	88.89±2.940
55	HSD 13202	98.89± 1.111	86.67±1.925	87.78±2.940
56	HSD 5463	92.22± 6.186	71.11±4.006	40.00±13.472
57	HSD 4928	96.67± 1.925	75.56±2.222	52.22±6.186
58	HSD 5479	86.67± 3.849	54.44±7.286	37.78±5.556
59	HSD 5461	98.89± 1.111	81.11±1.111	72.22±8.012
60	HSD 5901	88.89± 5.556	64.44±12.814	26.67±0.000

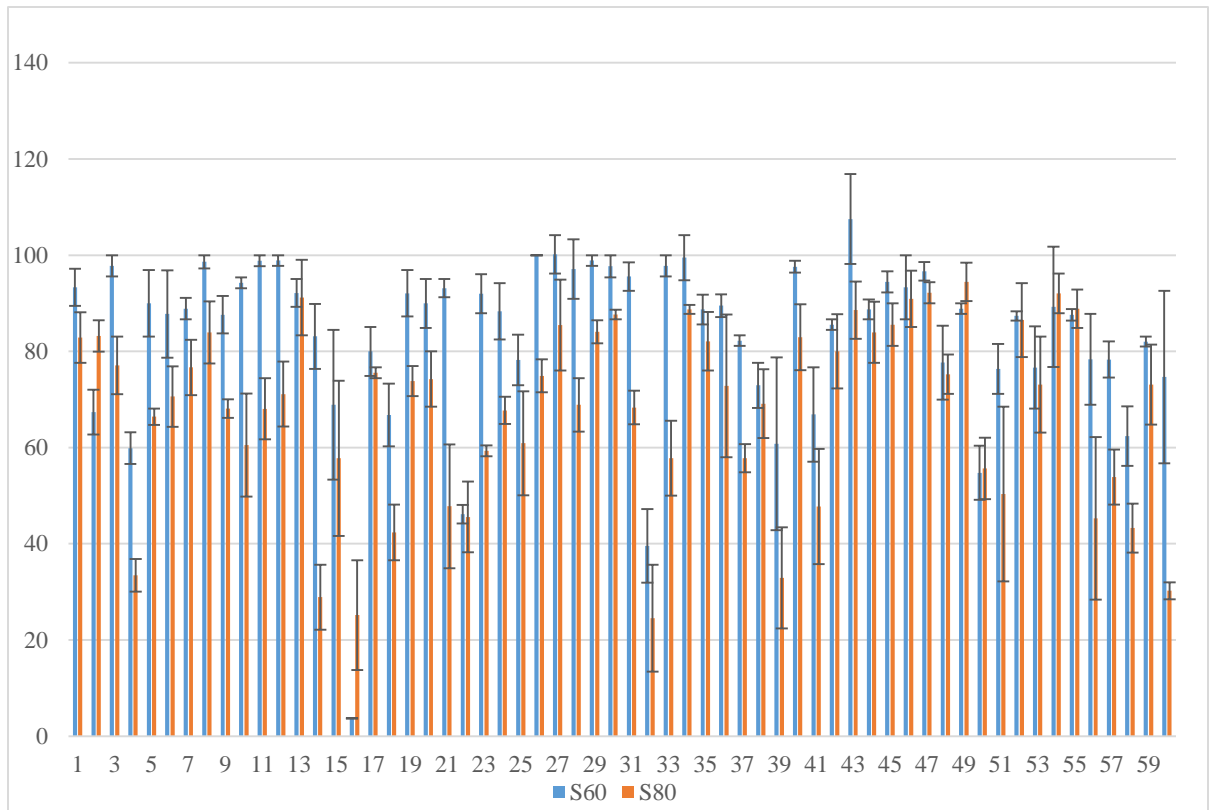


Figure 3.3 Effect of salinity stress Sodium chloride concentration(mM) on Sesame germination

3.3.2 Drought stress

3.3.2.1 Effect of drought stress Polyethylene glycol (bar) on Sesame seedlings growth shoot length (cm)

Table (3.3) figure (3.4) shows the effect of drought stress as poly ethylene glycol in sesame seedling shoot length. The treatments significantly ($P \leq 0.05$) decreased shoot length as compared to the control increasing poly ethylene glycol from zero to 6 bar showed non-significant differences while increasing the concentration up to 9 bar significantly decreased the seedling shoot length. The shoot length ranged between (1.53 ± 0.027 - 0.61 ± 0.173) cm at 6 bar and between (1.22 ± 0.080 - 0.32 ± 0.060) cm at 9 bar. The different between the varieties regarding the shoot length any concentration was attributed to the origin of the seeds. Increasing the drought stress from 6 to 9 bar decreased the shoot length.

3.3.2.2 Effect of drought stress on Sesame seedlings growth root length (cm)

As shown in table (3.4) figure (3.5) the seedling root length how the same pattern as the shoot length that, significant differences were observed between the treatment. With significant decreased in the root length at 9 bar. The root length ranged between (1.13 ± 0.071 - 0.07 ± 0.027) cm at 6 bar and between (1.03 ± 0.067 - 0.14 ± 0.020) cm at 9 bar. The different between the varieties regarding the root length and any concentration was attributed to the origin of the seeds. Increasing the drought stress from 6 to 9 bar decreased the root length.

These results were compatible with (Mensah *et al.* 2006) who reported that using varying PEG concentrations simulate drought effect on germination of

sesame and found that high concentration reduced the germination rate, radical and shoot development significantly, however, lower concentration could enhance root growth. Significantly reduced the percentage germination, radical and shoot development. Moreover, in sesame, several studies have reported that many traits including germination rate, seedling growth, shoot length and root length could be affect by drought stress (Liang *et al.* 2021). Turk *et al.* (2004) found that low level of drought stress hadn't any significant effect on germination, by increasing levels of drought germination and seedling growth reduced.

Table 3.3 : Effect of drought stress Polyethylene glycol (bar) on shoot growth of Sesame seedlings

	Accession No.	Shoot length (cm)		
		D0	D6	D9
1	HSD 4596	1.56±0.006	1.53±0.027	0.85±0.275
2	HSD 5464	1.42±0.122	1.05±0.287	0.35±0.073
3	HSD 3623	1.56±0.261	1.25±0.407	0.80±0.180
4	HSD 6697	1.61±0.127	0.75±0.295	1.05±0.107
5	HSD 3628	1.80±0.058	1.09±0.307	0.52±0.040
6	HSD 5453	1.22±0.028	0.73±0.193	0.84±0.357
7	HSD 4607	1.24±0.012	0.92±0.260	0.94±0.040
8	HSD 4603	1.14±0.012	1.01±0.107	1.06±0.080
9	HSD 4623	1.63±0.173	1.29±0.347	0.82±0.300
10	HSD 6679	1.57±0.067	1.19±0.293	0.59±0.207
11	HSD13194	1.69±0.047	0.80±0.280	0.80±0.300
12	HSD 4626	1.65±0.029	0.97±0.287	0.88±0.020
13	HSD13291	1.04±0.020	0.65±0.267	0.51±0.190
14	HSD 5472	1.33±0.088	0.87±0.207	0.68±0.283
15	HSD 3626	1.51±0.228	0.66±0.280	0.53±0.210
16	HSD 6770	1.41±0.125	1.03±0.333	1.17±0.007
17	HSD13264	1.28±0.101	1.07±0.053	0.79±0.013
18	HSD 3620	0.95±0.024	0.88±0.000	0.44±0.163
19	HSD13304	1.24±0.069	1.13±0.160	0.81±0.047
20	HSD 13697	1.25±0.192	0.85±0.121	0.50±0.197
21	HSD 5899	1.49±0.164	1.15±0.147	0.81±0.033
22	HSD 5457	1.13±0.081	1.08±0.099	0.50±0.203
23	HSD 13214	1.35±0.153	0.85±0.033	0.75±0.013
24	HSD 5471	1.55±0.107	1.17±0.427	0.75±0.227
25	HSD 4930	1.16±0.012	0.61±0.173	0.70±0.240
26	HSD 5455	1.50±0.058	0.95±0.133	0.45±0.173
27	HSD 13203	1.17±0.013	0.94±0.240	0.57±0.153
28	HSD 5888	1.51±0.070	1.13±0.033	0.30±0.100
29	HSD 13208	1.39±0.079	0.99±0.047	0.60±0.180
30	HSD 13251	1.04±0.012	0.81±0.073	0.74±0.040

continue

Sample number	Accession No.	Shoot length (cm)		
		D0	D6	D9
31	HSD13711	1.01±0.214	0.72±0.160	0.54±0.180
32	HSD11464	1.45±0.167	0.82±0.240	0.32±0.060
33	HSD13258	1.23±0.181	1.30±0.540	0.55±0.127
34	HSD13809	1.26±0.009	0.79±0.287	0.51±0.127
35	HSD13639	1.30±0.115	1.06±0.040	0.84±0.080
36	HSD11425	1.24±0.050	0.70±0.180	0.61±0.253
37	HSD 5451	1.15±0.085	0.99±0.107	0.81±0.087
38	HSD 6705	1.31±0.221	0.83±0.213	0.75±0.233
39	HSD 6728	1.47±0.117	1.19±0.247	0.88±0.240
40	HSD13682	1.29±0.159	1.10±0.240	0.88±0.240
41	HSD 5467	1.36±0.111	0.83±0.347	0.42±0.000
42	HSD11463	1.50±0.180	1.15±0.507	0.66±0.283
43	HSD13280	1.77±0.088	1.14±0.460	0.70±0.260
44	HSD13204	1.60±0.153	1.27±0.033	0.88±0.060
45	HSD11401	1.60±0.270	1.17±0.033	0.95±0.083
46	HSD13305	1.11±0.059	0.87±0.195	0.45±0.053
47	HSD 3619	1.65±0.187	1.01±0.413	0.65±0.273
48	HSD 4593	1.40±0.037	1.11±0.213	0.97±0.147
49	HSD 4599	1.11±0.035	0.82±0.200	0.65±0.127
50	HSD 6696	1.19±0.070	0.82±0.220	0.47±0.127
51	HSD 5462	1.41±0.137	1.37±0.133	1.22±0.080
52	HSD 3621	1.79±0.013	1.23±0.524	0.93±0.213
53	HSD 5448	1.04±0.023	0.89±0.155	0.57±0.127
54	HSD 5452	1.30±0.053	1.07±0.053	0.73±0.053
55	HSD 13202	1.24±0.193	0.97±0.393	0.87±0.113
56	HSD 5463	1.21±0.140	1.12±0.140	0.61±0.253
57	HSD 4928	1.01±0.134	0.77±0.073	0.37±0.033
58	HSD 5479	1.61±0.271	1.15±0.293	0.61±0.167
59	HSD 5461	1.21±0.066	1.02±0.010	0.76±0.100
60	HSD 5901	1.37±0.123	1.34±0.120	0.68±0.040

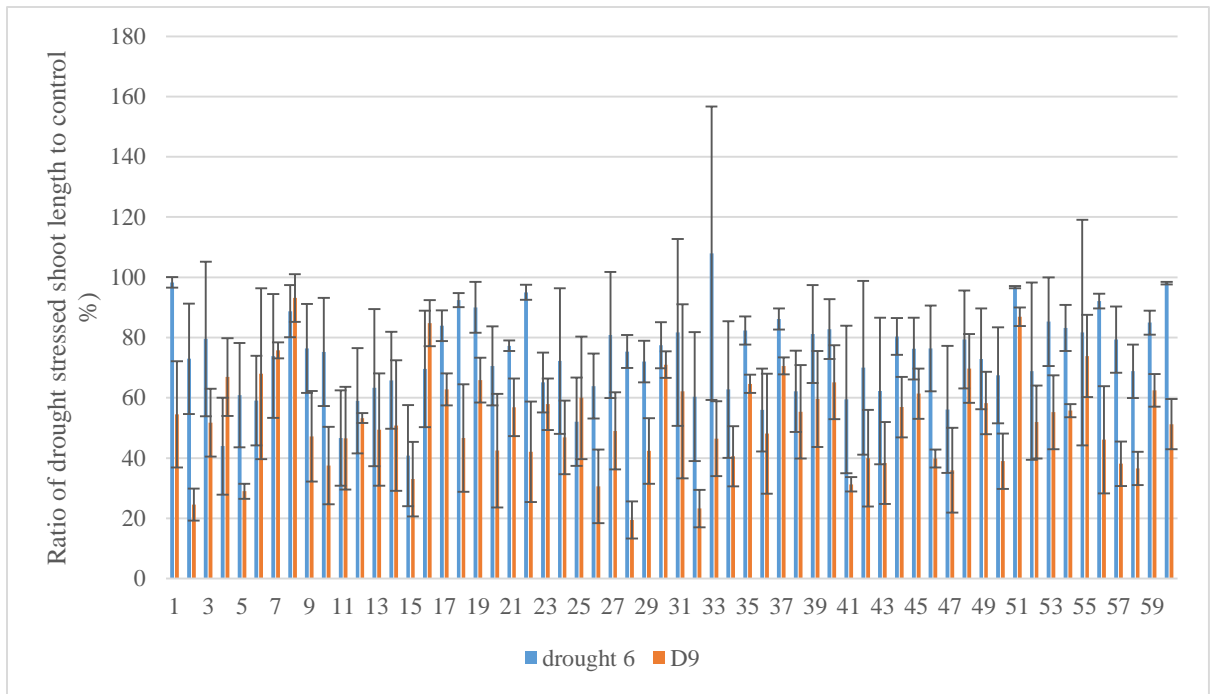


Figure 3.4 Effect of drought stress Polyethylene glycol (bar) on shoot growth of Sesame seedlings

Table 3.4: Effect of drought stress Polyethylene glycol (bar) on root growth of Sesame seedlings

Sample number	Accession No.	Root length (cm)		
		D0	D6	D9
1	HSD 4596	1.01± 0.048	0.77±0.127	0.67±0.273
2	HSD 5464	0.93± 0.154	0.83±0.118	0.14±0.020
3	HSD 3623	1.00 ± 0.095	1.02±0.230	0.71±0.267
4	HSD 6697	1.20± 0.265	0.71±0.113	0.57±0.113
5	HSD 3628	0.90± 0.208	0.73±0.163	0.30±0.083
6	HSD 5453	0.93± 0.044	0.66±0.110	0.56±0.203
7	HSD 4607	0.81± 0.029	0.70±0.050	0.51±0.033
8	HSD 4603	1.13± 0.135	0.62±0.060	0.23±0.087
9	HSD 4623	1.06± 0.076	0.51±0.013	0.49±0.210
10	HSD 6679	0.99± 0.113	0.89±0.067	0.40±0.163
11	HSD13194	0.88± 0.061	0.75±0.081	0.63±0.233
12	HSD 4626	0.84± 0.012	0.60±0.200	0.71±0.227
13	HSD13291	0.84±0.160	0.59±0.082	0.22±0.077
14	HSD 5472	1.20±0.129	0.80±0.180	0.51±0.227
15	HSD 3626	0.91±0.079	0.61±0.007	0.31±0.127
16	HSD 6770	0.99±0.391	0.07±0.027	0.63±0.047
17	HSD13264	1.11±0.071	0.57±0.093	0.29±0.073
18	HSD 3620	0.59±0.007	0.47±0.094	0.18±0.057
19	HSD13304	1.04±0.095	0.50±0.035	0.43±0.053
20	HSD 13697	1.20±0.306	0.55±0.103	0.20±0.063
21	HSD 5899	0.97±0.013	0.69±0.093	0.34±0.040
22	HSD 5457	1.02±0.020	0.43±0.033	0.23±0.073
23	HSD 13214	1.00±0.000	0.52±0.110	0.41±0.067
24	HSD 5471	1.09±0.007	0.56±0.020	0.41±0.087
25	HSD 4930	0.56±0.145	0.79±0.147	0.46±0.140
26	HSD 5455	0.98±0.042	0.73±0.133	0.82±0.163
27	HSD 13203	0.87±0.024	0.64±0.122	0.30±0.140
28	HSD 5888	1.01±0.013	0.71±0.167	0.63±0.270
29	HSD 13208	1.13±0.033	0.80±0.120	0.33±0.153
30	HSD 13251	0.81±0.073	0.35±0.013	0.25±0.027

Continue

Sample number	Accession No.	Root length (cm)		
		D0	D6	D9
31	HSD13711	0.71±0.047	0.59±0.147	0.18±0.040
32	HSD11464	1.18±0.133	0.53±0.018	0.17±0.030
33	HSD13258	1.05±0.068	0.94±0.020	0.23±0.047
34	HSD13809	1.07±0.066	0.73±0.013	0.22±0.043
35	HSD13639	1.13±0.077	0.79±0.213	0.33±0.047
36	HSD11425	0.85±0.044	0.92±0.080	0.47±0.190
37	HSD 5451	0.97±0.044	0.83±0.033	0.53±0.033
38	HSD 6705	1.27±0.037	0.69±0.047	0.49±0.170
39	HSD 6728	1.01±0.007	0.42±0.072	0.72±0.220
40	HSD13682	0.93±0.037	0.63±0.127	0.59±0.210
41	HSD 5467	0.86±0.083	0.47±0.090	0.17±0.013
42	HSD11463	1.27±0.052	1.13±0.071	0.67±0.033
43	HSD13280	0.94±0.031	0.45±0.037	0.44±0.120
44	HSD13204	0.99±0.087	0.71±0.096	0.47±0.007
45	HSD11401	0.87±0.029	0.81±0.047	0.75±0.127
46	HSD13305	0.94±0.031	0.65±0.035	0.19±0.013
47	HSD 3619	1.16±0.106	1.01±0.007	0.49±0.153
48	HSD 4593	0.96±0.072	0.77±0.079	0.63±0.193
49	HSD 4599	1.23±0.027	1.01±0.193	0.20±0.023
50	HSD 6696	1.04±0.040	0.64±0.140	0.22±0.040
51	HSD 5462	1.30±0.058	0.63±0.033	0.88±0.009
52	HSD 3621	1.77±0.145	0.73±0.093	1.03±0.067
53	HSD 5448	1.03±0.033	0.42±0.040	0.22±0.040
54	HSD 5452	1.10±0.042	0.50±0.080	0.37±0.070
55	HSD 13202	1.19±0.013	0.37±0.007	0.46±0.160
56	HSD 5463	1.09±0.087	0.32±0.114	0.43±0.170
57	HSD 4928	0.96±0.151	0.43±0.064	0.44±0.120
58	HSD 5479	0.81±0.098	0.35±0.018	0.33±0.093
59	HSD 5461	0.95±0.024	0.53±0.064	0.75±0.147
60	HSD 5901	1.01±0.007	0.89±0.113	0.65±0.033

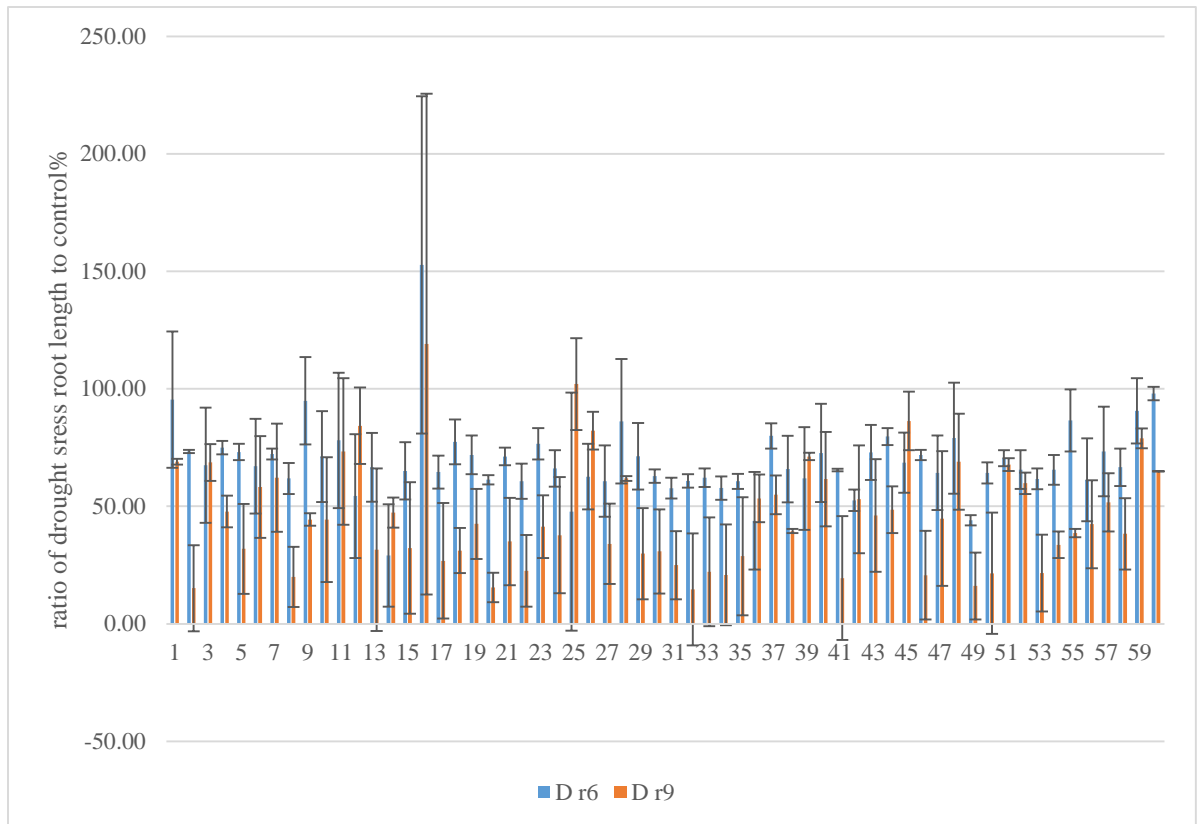


Figure 3.5 Effect of drought stress Polyethylene glycol (bar) on root growth of Sesame seedlings

3.3.3 Salinity stress

3.3.3.1 Effect of salinity stress on Sesame seedlings growth shoot length (cm)

The salinity stress Sodium chloride concentration at (0, 60 and 80) mM affected the sesame seedlings shoot length significantly ($P \leq 0.05$) difference was observed between the varieties compared to the control as shown in Table (3.5) and figure (3.6). The shoot length ranged between (1.50 ± 0.040 _ 0.29 ± 0.059) at 60 mM and between (1.27 ± 0.530 _ 0.41 ± 0.208) at 80 mM. The different between the varieties regarding the shoot length and any concentration was attributed to the origin of the seeds, Increasing the salinity stress from (60 and 80) mM decreased the shoot length.

3.3.3.2 Effect of salinity stress on Sesame seedlings growth root length (cm)

Table (3.6) and figure (3.7) shows the effect of salinity stress Sodium chloride concentration (0, 60 and 80) mM on sesame seedlings root length significant ($P \leq 0.05$) decreased was observed between the varieties compared to the control. The root length ranged between (1.13 ± 0.071 _ 0.07 ± 0.027) at 60 mM and between (1.15 ± 0.167 _ 0.03 ± 0.003) at 80 mM. The different between the varieties regarding the root length and any concentration was attributed to the origin of the seeds, Increasing the salinity stress from (60 and 80) mM decreased the root length.

The same result was attained by Bekele *et al.* 2017 who observed that increasing the concentration of NaCl decrease seedling growth shoot and root length. (Bazrafshan and Ehsanzadeh 2014) mentioned that Increasing NaCl concentration decrease in the net photosynthetic rate, stomatal conductance

salinity. Moreover, Adamu and Abu-Bakr, 2018 Obtained the same results. Mensah et al. 2006 significantly reduced the shoot development to increase sodium chloride.(Desingh and Kanagaraj 2020) Suassuna *et al.* 2017, Heydari *et al.* 2019 and Mamo et al. 2021 Similarly, for seedling rate were decreased for root length, shoot length to increase sodium chloride concentrations.

Table 3.5 : Effect of salinity stress Sodium chloride concentration (mM) on shoot growth of Sesame Seedlings

Sample number	Accession No.	Shoot length (cm)		
		S0	S60	S80
1	HSD 4596	1.56±0.006	1.46±0.012	0.86±0.117
2	HSD 5464	1.42±0.122	1.23±0.018	1.15±0.047
3	HSD 3623	1.56±0.261	1.23±0.233	0.90±0.060
4	HSD 6697	1.61±0.127	1.13±0.053	0.44±0.191
5	HSD 3628	1.80±0.058	1.35±0.037	0.96±0.040
6	HSD 5453	1.22±0.028	1.11±0.007	0.41±0.208
7	HSD 4607	1.24±0.012	1.05±0.073	0.56±0.178
8	HSD 4603	1.14±0.012	1.13±0.007	0.55±0.174
9	HSD 4623	1.63±0.173	1.13±0.073	0.99±0.033
10	HSD 6679	1.57±0.067	1.37±0.070	0.57±0.153
11	HSD13194	1.69±0.047	1.21±0.177	0.55±0.187
12	HSD 4626	1.65±0.029	1.50±0.040	0.84±0.120
13	HSD13291	1.04±0.020	1.01±0.006	0.87±0.013
14	HSD 5472	1.33±0.088	1.07±0.007	0.65±0.117
15	HSD 3626	1.51±0.228	1.15±0.007	0.65±0.098
16	HSD 6770	1.41±0.125	0.29±0.059	0.51±0.226
17	HSD13264	1.28±0.101	1.01±0.033	0.79±0.059
18	HSD 3620	0.95±0.024	0.92±0.023	0.48±0.181
19	HSD13304	1.24±0.069	0.95±0.018	0.87±0.007
20	HSD 13697	1.25±0.192	1.10±0.031	0.71±0.166
21	HSD 5899	1.49±0.164	0.93±0.007	0.75±0.033
22	HSD 5457	1.13±0.081	0.92±0.031	0.77±0.103
23	HSD 13214	1.35±0.153	0.96±0.080	0.83±0.087
24	HSD 5471	1.55±0.107	0.98±0.060	0.74±0.180
25	HSD 4930	1.16±0.012	1.13±0.007	0.85±0.057
26	HSD 5455	1.50±0.058	1.24±0.103	0.93±0.007
27	HSD 13203	1.17±0.013	0.99±0.055	0.94±0.031
28	HSD 5888	1.51±0.070	0.97±0.027	0.80±0.100
29	HSD 13208	1.39±0.079	1.41±0.027	0.91±0.018
30	HSD 13251	1.04±0.012	0.95±0.044	0.69±0.177

Continue

Sample number	Accession No.	Shoot length (cm)		
		S0	S60	S80
31	HSD13711	1.01±0.214	1.07±0.077	0.53±0.057
32	HSD11464	1.45±0.167	1.01±0.103	0.47±0.058
33	HSD13258	1.23±0.181	1.07±0.053	0.85±0.107
34	HSD13809	1.26±0.009	1.27±0.013	0.78±0.100
35	HSD13639	1.30±0.115	1.21±0.193	0.89±0.105
36	HSD11425	1.24±0.050	1.39±0.207	0.79±0.123
37	HSD 5451	1.15±0.085	1.10±0.040	0.79±0.048
38	HSD 6705	1.31±0.221	1.02±0.031	0.83±0.113
39	HSD 6728	1.47±0.117	0.95±0.027	0.76±0.083
40	HSD13682	1.29±0.159	1.14±0.100	0.89±0.007
41	HSD 5467	1.36±0.111	0.94±0.031	0.66±0.172
42	HSD11463	1.50±0.180	1.43±0.167	1.08±0.120
43	HSD13280	1.77±0.088	0.91±0.018	0.67±0.185
44	HSD13204	1.60±0.153	1.03±0.077	0.81±0.070
45	HSD11401	1.60±0.270	1.34±0.000	0.93±0.047
46	HSD13305	1.11±0.059	1.24±0.220	0.92±0.040
47	HSD 3619	1.65±0.187	1.33±0.067	0.93±0.007
48	HSD 4593	1.40±0.037	1.11±0.116	0.81±0.027
49	HSD 4599	1.11±0.035	1.05±0.000	0.89±0.007
50	HSD 6696	1.19±0.070	0.93±0.033	0.91±0.027
51	HSD 5462	1.41±0.137	1.00±0.000	0.89±0.059
52	HSD 3621	1.79±0.013	1.15±0.027	0.91±0.013
53	HSD 5448	1.04±0.023	0.97±0.041	0.81±0.121
54	HSD 5452	1.30±0.053	1.01±0.093	0.93±0.007
55	HSD 13202	1.24±0.193	0.93±0.107	0.92±0.000
56	HSD 5463	1.21±0.140	0.64±0.012	0.76±0.223
57	HSD 4928	1.01±0.134	0.93±0.041	0.81±0.107
58	HSD 5479	1.61±0.271	0.91±0.055	0.67±0.181
59	HSD 5461	1.21±0.066	0.91±0.047	1.27±0.530
60	HSD 5901	1.37±0.123	1.28±0.120	0.84±0.061

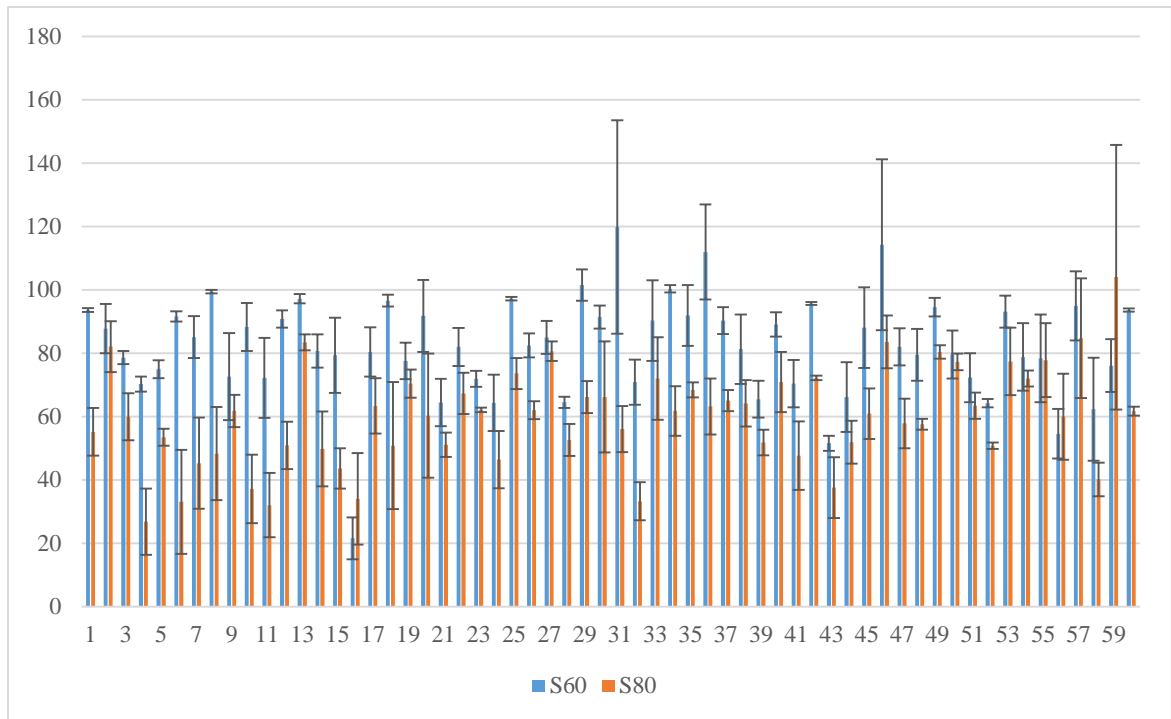


Figure 3.6 Effect of salinity stress Sodium chloride concentration (mM) on shoot growth of Sesame Seedlings

Table 3.6: Effect of salinity stress Sodium chloride concentration (mM) on root growth of Sesame seedlings

Sample number	Accession No.	Root length (cm)		
		S0	S60	S80
1	HSD 4596	1.01± 0.048	0.77±0.127	0.51±0.100
2	HSD 5464	0.93± 0.154	0.83±0.118	1.15±0.167
3	HSD 3623	1.00 ± 0.095	1.02±0.230	0.38±0.040
4	HSD 6697	1.20± 0.265	0.71±0.113	0.15±0.093
5	HSD 3628	0.90± 0.208	0.73±0.163	0.36±0.020
6	HSD 5453	0.93± 0.044	0.66±0.110	0.20±0.087
7	HSD 4607	0.81± 0.029	0.70±0.050	0.43±0.350
8	HSD 4603	1.13± 0.135	0.62±0.060	0.19±0.104
9	HSD 4623	1.06± 0.076	0.51±0.013	0.52±0.080
10	HSD 6679	0.99± 0.113	0.89±0.067	0.19±0.110
11	HSD13194	0.88± 0.061	0.75±0.081	0.21±0.121
12	HSD 4626	0.84± 0.012	0.60±0.200	0.44±0.080
13	HSD13291	0.84±0.160	0.59±0.082	0.33±0.168
14	HSD 5472	1.20±0.129	0.80±0.180	0.25±0.041
15	HSD 3626	0.91±0.079	0.61±0.007	0.32±0.042
16	HSD 6770	0.99±0.391	0.07±0.027	0.03±0.003
17	HSD13264	1.11±0.071	0.57±0.093	0.43±0.081
18	HSD 3620	0.59±0.007	0.47±0.094	0.14±0.081
19	HSD13304	1.04±0.095	0.50±0.035	0.62±0.040
20	HSD 13697	1.20±0.306	0.55±0.103	0.29±0.074
21	HSD 5899	0.97±0.013	0.69±0.093	0.30±0.020
22	HSD 5457	1.02±0.020	0.43±0.033	0.29±0.044
23	HSD 13214	1.00±0.000	0.52±0.110	0.33±0.055
24	HSD 5471	1.09±0.007	0.56±0.020	0.39±0.097
25	HSD 4930	0.56±0.145	0.79±0.147	0.31±0.067
26	HSD 5455	0.98±0.042	0.73±0.133	0.44±0.050
27	HSD 13203	0.87±0.024	0.64±0.122	0.61±0.157
28	HSD 5888	1.01±0.013	0.71±0.167	0.49±0.105
29	HSD 13208	1.13±0.033	0.80±0.120	0.31±0.007
30	HSD 13251	0.81±0.073	0.35±0.013	0.37±0.109

Continue

Sample number	Accession No.	Root length (cm)		
		S0	S60	S80
31	HSD13711	0.71±0.047	0.59±0.147	0.21±0.024
32	HSD11464	1.18±0.133	0.53±0.018	0.16±0.012
33	HSD13258	1.05±0.068	0.94±0.020	0.41±0.073
34	HSD13809	1.07±0.066	0.73±0.013	0.43±0.101
35	HSD13639	1.13±0.077	0.79±0.213	0.41±0.027
36	HSD11425	0.85±0.044	0.92±0.080	0.32±0.012
37	HSD 5451	0.97±0.044	0.83±0.033	0.55±0.225
38	HSD 6705	1.27±0.037	0.69±0.047	0.46±0.120
39	HSD 6728	1.01±0.007	0.42±0.072	0.31±0.055
40	HSD13682	0.93±0.037	0.63±0.127	0.46±0.040
41	HSD 5467	0.86±0.083	0.47±0.090	0.26±0.076
42	HSD11463	1.27±0.052	1.13±0.071	0.71±0.207
43	HSD13280	0.94±0.031	0.45±0.037	0.29±0.070
44	HSD13204	0.99±0.087	0.71±0.096	0.38±0.092
45	HSD11401	0.87±0.029	0.81±0.047	0.55±0.093
46	HSD13305	0.94±0.031	0.65±0.035	0.57±0.033
47	HSD 3619	1.16±0.106	1.01±0.007	0.48±0.040
48	HSD 4593	0.96±0.072	0.77±0.079	0.55±0.087
49	HSD 4599	1.23±0.027	1.01±0.193	0.51±0.033
50	HSD 6696	1.04±0.040	0.64±0.140	0.34±0.000
51	HSD 5462	1.30±0.058	0.63±0.033	0.39±0.024
52	HSD 3621	1.77±0.145	0.73±0.093	0.37±0.024
53	HSD 5448	1.03±0.033	0.42±0.040	0.32±0.060
54	HSD 5452	1.10±0.042	0.50±0.080	0.64±0.140
55	HSD 13202	1.19±0.013	0.37±0.007	0.43±0.052
56	HSD 5463	1.09±0.087	0.32±0.114	0.30±0.081
57	HSD 4928	0.96±0.151	0.43±0.064	0.35±0.048
58	HSD 5479	0.81±0.098	0.35±0.018	0.31±0.077
59	HSD 5461	0.95±0.024	0.53±0.064	0.29±0.024
60	HSD 5901	1.01±0.007	0.89±0.113	0.33±0.029

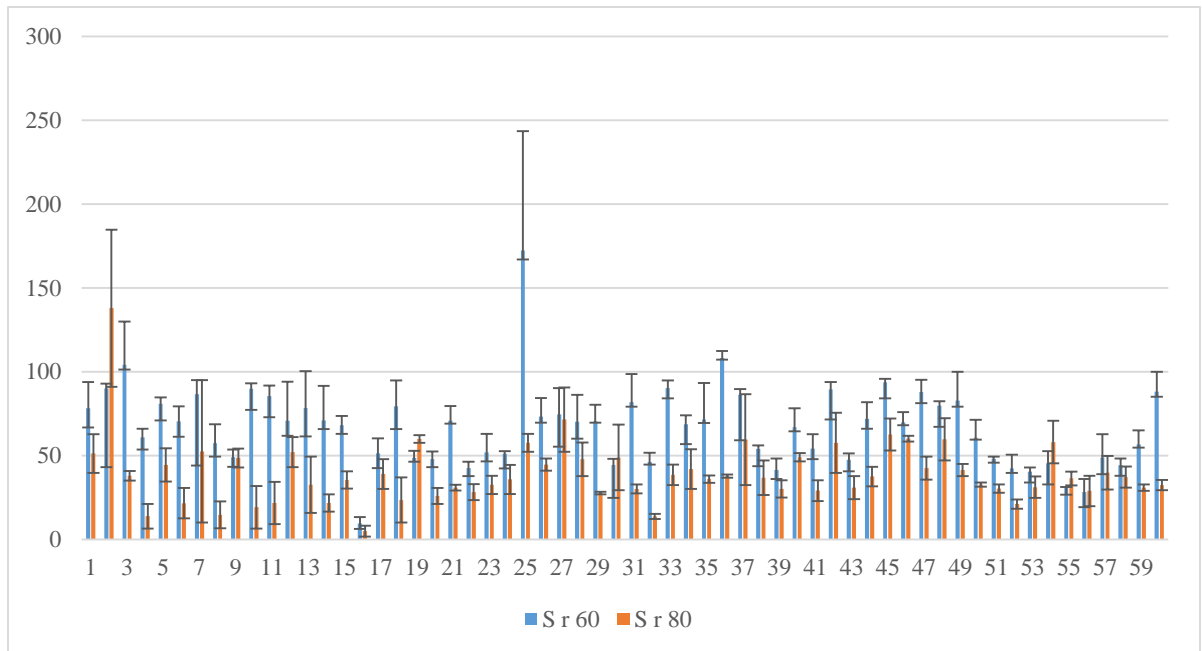


Figure 3.7 *Effect of salinity stress Sodium chloride concentration (mM) on root growth of Sesame seedlings*

3.3.4 Stander germination test (SGT)

Figuer (3.8) showed the total germination percentage (TG), normal and abnormal germination percentage (NS and AB) respictivly frequency in affected by drought and salinity stress (6 (dt1) -9 (dt2)) bar and (60 (sl1) -80 (sl2))mM respictivly compared to the control(CT) at 20, 40, 60, 80 and 100% seed germination.

The total germination percentage ranged between 0 and 100% in the control untreated seeds, within this range more than 80% showed anormal germination seeds.(3.8.A)

Drought stress decreased the percentage range of germination from zero to 60% , Increasing the drought stress from 6 to 9 bar has no different in the total germination percentage .the drought stress resulted in abnormal seed germination by more than 65% of the total germinate seed with both drought concentration.(3.8.B)

The range of germination percentage as affected by salinity stress decreased showing arange of zero -23% (80mM) with asignificant diffrence between 60-80 mM concetration, as the germination percentage decreased by more than 50% with increasing salinity conc. Increasing the salinity resulted in adrastic abnormal seed germination resuling in more than 90%of abnormal seed germination.(3.8.C)

The frequncy of shoot length (SL), root length (RL) and root to shoot ratio (RS) as affected by drought and salinity stress (6 (dt1) -9 (dt2)) bar and (60 (sl1) -80 (sl2)) mM respictivly compared to the control(CT) at 20, 40, 60, 80 and 100% seed germination was ellistrated in figure (3.9). all most all the tested sesame germinated seed showed 100% growth with maxium seeds

shoot length with the control untreated seeds. The seed germination was affected by drought stress as 50% of the seeds showing 80% of shoot length, increasing the drought (dt1 to dt2) decreased formation of the shoot where 40% of germinated seeds possess a maximum growth of 60% shoot length.

As the drought, salinity stress follows the same trend, increasing the salinity concentration decreased the percentage of the shoot growth. From 80 to 100% of the seeds reached a maximum of 90% growth compared to (60-80%) of the seeds reaching 70% of maximum shoot length figure (3.9.A).

All the tested 60 cultivars reached the maximum shoot length frequency at all most 100%. 70% frequency of the cultivars have a root length 80% percentage at the drought concentration (dt1) compared to 50% shoot length. About 60% of the cultivars have about (40-60)% root growth at (dt2).

53% of cultivars reached a maximum root length at 60% germination with increasing the concentration to (sl2) resulted in decreasing the frequency of root length at 80 and 100% with the maximum of more than 50% of the cultivars showing 40% of root length in (3.9.B).

Figure (3.9.C) showed the root to shoot ratio as calculated by $\text{root/shoot} \times 100\%$ in all the five treatments.

Germination had been recorded daily for 5 days and data were used to analyse germination speed i.e. the time required to reach 10% germination (T10), the time required to 50% germination (T50) and the area under the germination time curve after 150 hours (AUC) figure (3.10).

Almost all the tested seed reached 10% germination in a time of about 135 hours (40%) from the beginning of the experiment. few variation were observed among the treatments compared to the control (3.10.A).

As in the T10 and T50 reached the maximum germination in (135-175) hours (40-60)% depending on the treatment. The variation in the time taken for the seed germination was attributed to sesame genotypes tested as observed in the field experiment(3.10.B).

The area under the curve (AUC) increased with increasing time regardless the treatments(3.10.C).

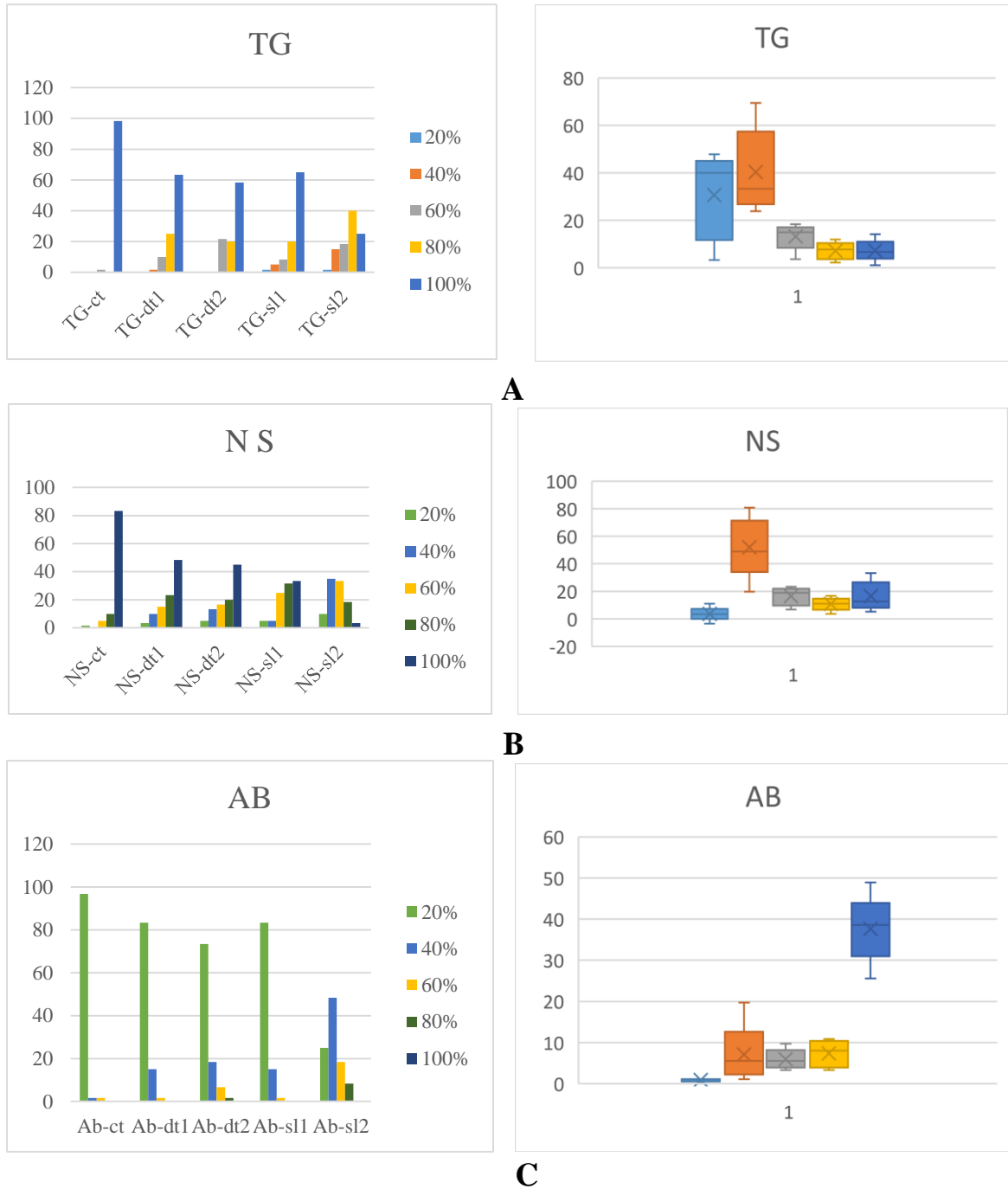
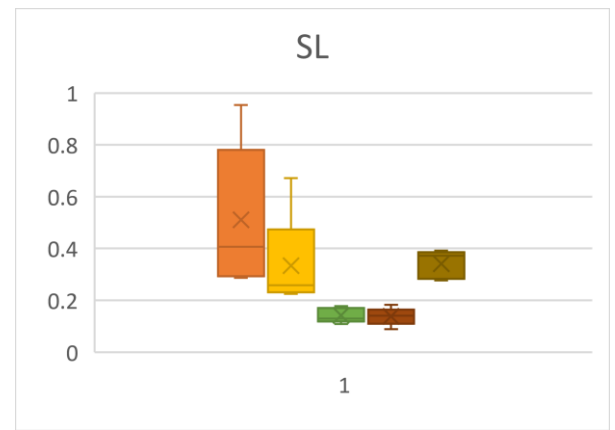
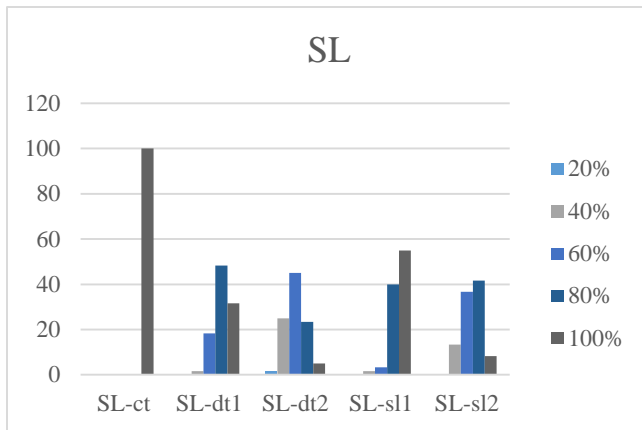
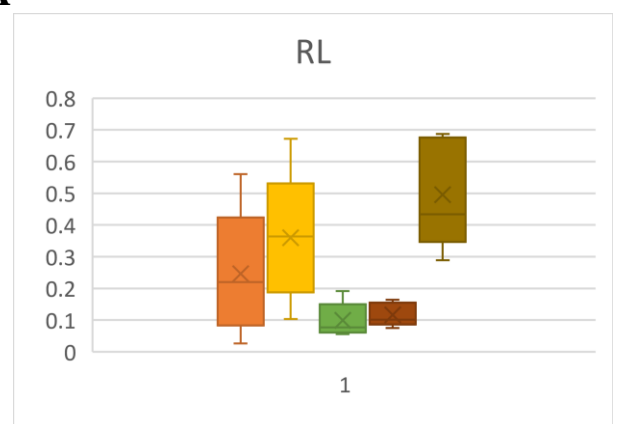
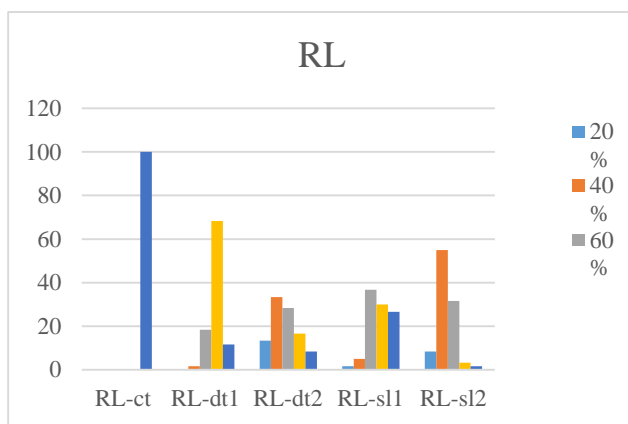


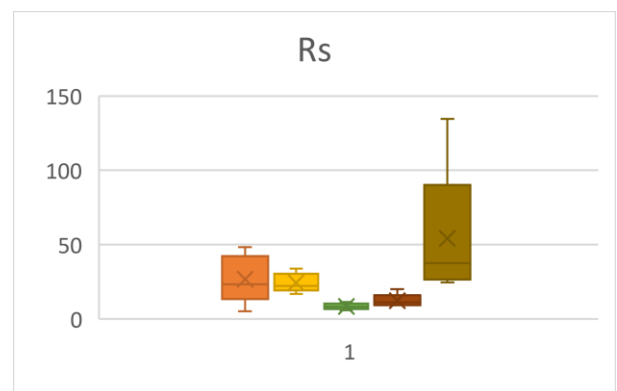
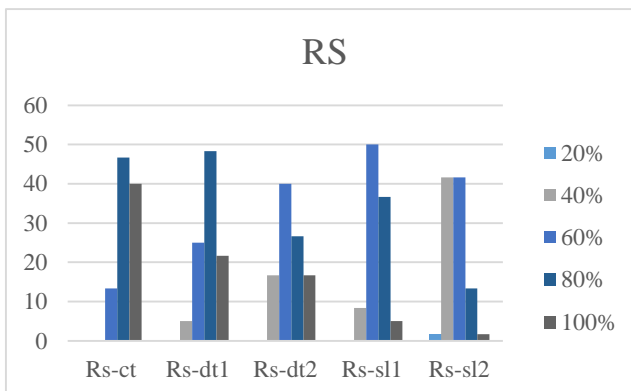
Figure 3.8 germination traits indicating the effect of drought and salinity stress on sesame cultivars, the range of seeds performance is shown by histogram (left) and box plot (right): total germination (TG), normal germination (NS) and abnormal (AB).



A

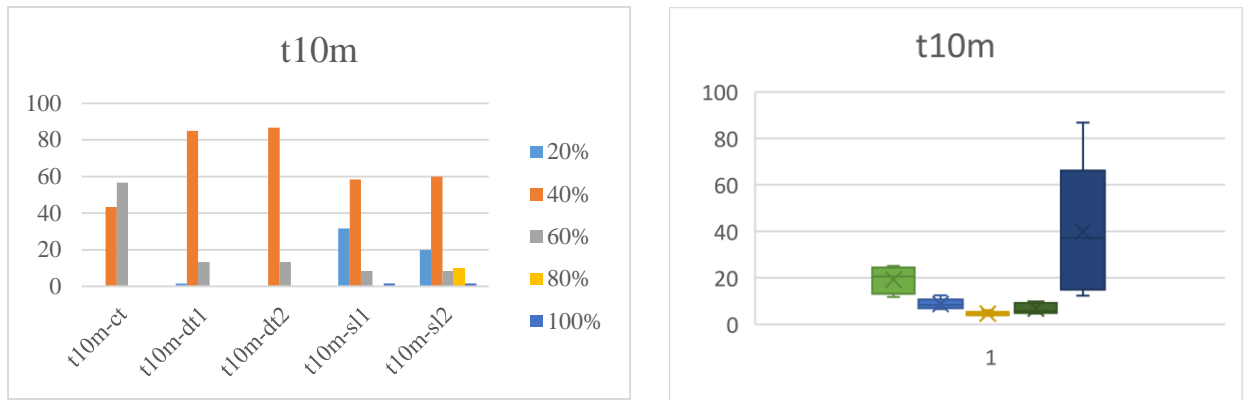


B

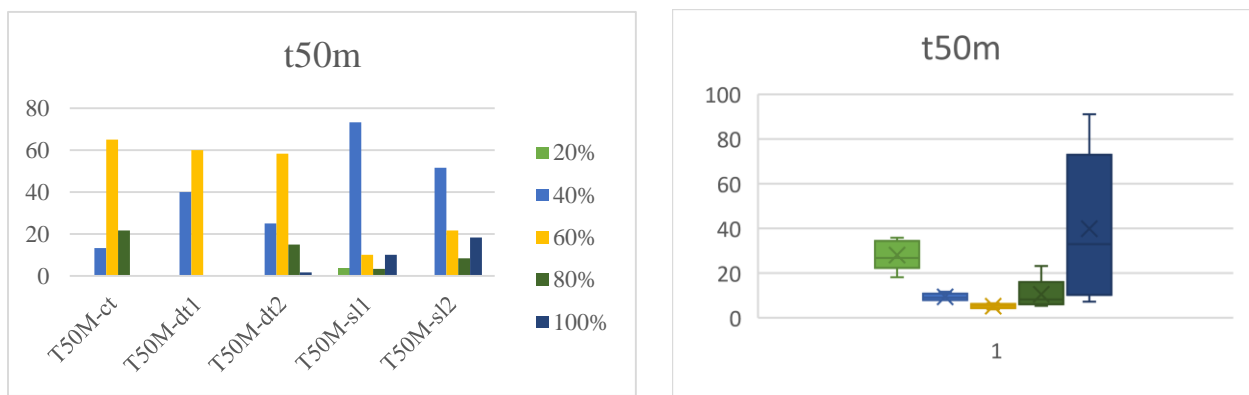


C

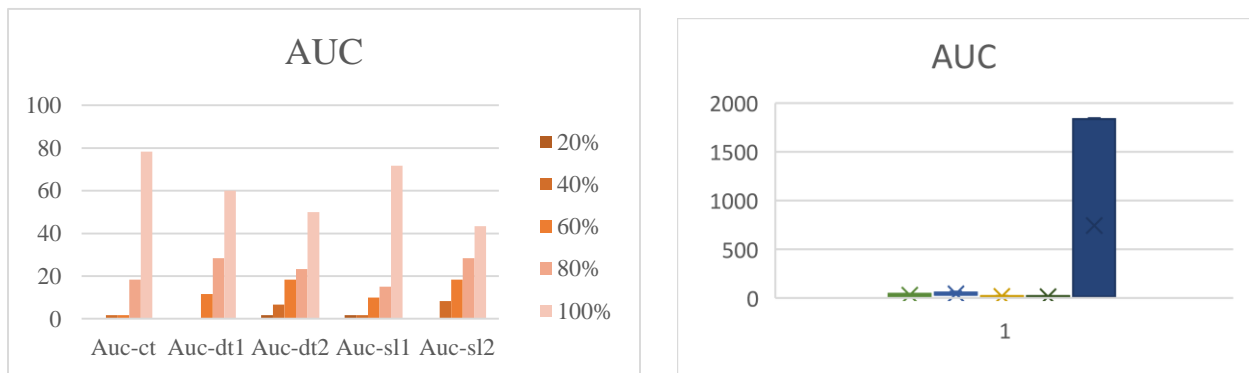
Figure 3.9 germination traits indicating the effect of drought and salinity stress on sesame cultivars, the range of seeds performance is shown by histogram (left) and box plot (right): shoot length cm (sl), root length cm (RL) and root shoot ratio cm (RS).



A



B



C

Figure 3.10 germination traits indicating the effect of salinity and drought stress on sesame cultivars , the range of seeds performance is shown by histogram (left) and box plot (right): time to 10% germination (T10m) ,time to 50% germination (T50m) area under the curve (AUC).

Chapter four

Genetic Diversity of Sesame Genotypes using Molecular Markers (ISSR)

Chapter four

4.1 Literature review (ISSR)

4.1.1 Origin and Distribution of Sesame

Sesame is a diploid species with $2n = 26$ chromosomes. The cultivated sesame (*Sesamum indicum* L.) is an annual plant that belongs to the Pedaliaceae family. It is considered as one of the oldest crops cultivated by man, having been grown in the Near East and Africa for over 5,000 years for cooking and medicinal purposes.

It is very rich in compounds like lignan, sesamin and sesamolin (Abdalla, 2017). All these properties show the nutritional significance and medicinal importance making it highly valued crop.

Although the origin of sesame is still in debate, some authors considered Ethiopia as the basic diversity center of cultivated sesame (Mahajan, *et al.* 2007). The genus has many species, and most are wild. Most wild species of the genus *Sesamum* are native to sub-Saharan Africa. *S. indicum*, the cultivated type (Bedigian 2004) originated in India. Sesame is cultivated in tropical and subtropical regions of Asia, Africa and Southern America. As most of these wild species are native to sub-Saharan Africa, sesame was originally believed to have originated in Africa; however, domesticated sesame has since been shown to have originated on the Indian sub-continent). Bedigian (2004) Further investigation into the evolution of sesame has been hampered by the absence of detailed molecular data across multiple sesame strains.

4.1.2 Economic Importance

Generally, 65% of world sesame production is used for edible oil extraction and 35% for confectionery purpose (Pham *et al.* 2009). The fatty acid composition is rather attractive, due to the high level of unsaturated fatty acids. Sesame is an important source of high quality edible oil and protein food for poor farmers of major sesame growing countries such as Sudan, Ethiopia, Nigeria, Uganda, Mexico, Venezuela, India, China, Pakistan, Turkey, and Myanmar who can hardly afford animal fat and protein. Sesame seed is the single readily available source of protein high in sulfur-containing amino acids (Morris 2002). It is the major cash crop for smallholder farmers and valuable foreign exchange revenue for the economy of different countries. The remaining cakes of sesame are used as a source of crude protein for cattle feed.

4.1.3 Genetic resources of sesame in Sudan

Genetic studies of important complex traits were started by the availability of diverse molecular markers, which allow the screening of variable alleles for advanced breeding programs. In Sudan some attempts were made in 1950's and early 1960's to collect landraces of sesame and many introductions were made from most sesame growing regions of the world between 1950 and 1980. These were used by plant breeders (FAO 2013). Since genetic base of cultivated sesame is narrow, genetic characterization plays a key role in sesame breeding programs. Because of the lack of proper storage facilities, sesame and groundnut collections were maintained by annual planting. This expensive method of maintenance was discontinued because of the scarcity of

funds and lower priority given to this activity by the breeders. Most of these collections were lost due to destruction by store pests and loss of viability.

4.1.4 Sesame cultivars

In Sudan sesame is annual crop grown in all parts of the Sudan, and in some regions on a considerable scale. There are many varieties and strains, which are known in sesame-growing countries, which differ considerably in size, form, and color of flowers, seed size, color and composition of seeds. Non-shattering cultivars are now being developed (Abate *et al.* 2015b). Several sesame varieties are used in the world market. They include local and commercial varieties. However, the cultivation of improved varieties is limited due to insufficient variety information. The farmers continue to grow local varieties with low yields (Pham *et al.* 2010).

A large amount of variations in days to flower initiation, plant growth, number of branches and yield components such as number of capsules, number of seeds per capsule, seed yield per plant exist between different sesame varieties (Negasa 2014).

In Sudan, generally, local varieties Hirihri (early maturing), Jabarouk (medium) and Baladi or Jaball (late maturing) are cultivated in Kordofan. Improved seed varieties are cultivated in limited area in mechanized Agricultural farms at Gadaref and Damazin due to un-availability of improved seed (Khidir 1997). Some varieties are released in the last 20 years ago, with variable seed colour. These include the white seeded varieties Zirra 1,3,7,9 and Kenana-1, and brown-seeded varieties Huria 1, 31 and 49. Another white-seeded variety namely Kenana2 was released in 1991, which now dominates

most of the central rain-lands (Ahmed 1998). Lately two varieties were released namely Khidir and Promo.

The sesame cultivar, Khidir, was released for improved production in high rainfall areas. It has a vigorous growth, dark green color, highly branching, medium maturity (90- 105 days), with white seeds, and high yielding (750- 1000 kg/ha). It is adapted to a wide range of environments. The average 1000 seed weight is 3.24 g. Its seed oil and protein contents are 49.0 and 30.6%, respectively.

Moreover, sesame cultivar Promo which has improves production in areas of high and low rainfall, has vigorous growth with light green color, medium branching. The cultivar is 150 cm tall, white seeded, medium yield (600- 800 kg/ha). It has a 1000 seed weight of 3.42 g and seed oil and protein contents of 48.0 and 31.0% respectively (Ahmed 1998).

4.1.5 Sesame genome

Sesame genome size, which is about 350 Mb, is not largely explored. A number of 27,148 genes have been annotated in a sesame reference genome that shows a low proportion of repetitive sequences (28.5%) (Wang, *et al.* 2014).

Genetic diversity in germplasm collections is a requirement for a breeding program to create high yielding sesame varieties. Molecular marker ISSR was patterns used of genetic variation cultivated sesame accessions.(Singh, *et al.* 2015). ISSR involved using micro-satellite sequence order that spread across the genome as primers in a polymerase chain reaction to produce multi-locus markers) (Parsaeian, *et al.* 2011). (Meriem, *et al.* (2021) reported studies of diversity in sesame over the world using ISSR.

4.1.6 Genetic variability and diversity

Genetic diversity of crops plays an important role in sustainable development and food security (Esquinas-Alcázar, 2005), as it allows the cultivation of crops in the presence of various biotic and abiotic stresses. Information on genetic diversity is important when working to improve crop varieties. It is also important for selection of parents that can be used in plant breeding programs. Genetic diversity is analyzed by using various methods such as morphological, biochemical and molecular markers. Genetic variability, which is due to the genetic differences among individuals within a population, is the core of plant breeding because proper management of diversity can produce permanent gain in the performance of plant and can buffer against seasonal fluctuations. In addition, estimation of the magnitude of variation within germplasm collections for important plant attributes will enable breeders to exploit genetic variability more efficiently (Jahufer and Gawler 2000).

Sesame is a rare example of non-model and minor crop for which numerous genetic loci and candidate genes underlying features of interest have been disclosed at relatively high resolution (Berhe, *et al.* 2021). The genetic diversity of sesame populations from different locations in Tadla was investigated using ISSR (Inter Sequence Simple Repeats) markers as a simple method to reveal polymorphism among them. A total of 130 individuals representing 31 populations were sampled. Twenty-four ISSR primers were used for analysis of individuals representing the 31 different sesame populations grown in different agro climatic (Meriem, *et al.* 2021). Cluster analysis based on morphological and molecular marker classified sesame genotypes into two major groups. Mantel test showed an insignificant correlation between phenotypic and molecular marker information. The

genotypes belonging to the same geographical area did not always occupy the same cluster. The results confirmed that both genetic and phenotypic diversity in a combined way could efficiently evaluate the variation present in different sesame accessions in any breeding program.(Pandey, *et al.* 2015).

Singh, *et al.* (2015) reported that molecular markers RAPD, ISSR and SSRs are highly discriminatory, precise and reproducible markers for studying genetic diversity analysis. The genotype and locality specific bands obtained through SSRs and ISSRs can be converted to gene specific primers which could effectively be used for the identification of genotypes, protection of intellectual property right and genetic purity analysis.

ISSR markers could detect the level of genetic diversity and group sesame collections. Most recently, ISSR markers and EST tags were developed for the creation of genetic maps in sesame (Parsaeian, *et al.* 2011). (Meriem, *et al.* 2021) reported that the genetic diversity of Moroccan sesame populations. Polymorphism and PIC found in this study indicated that the ISSR markers used were highly reproducible. Although a limited number of ISSR markers were used in the study, the results confirm that ISSR markers are effective in detecting polymorphism between Moroccan sesame populations. However, the high similarity coefficients found between them as well as the pairwise comparison of these populations show that there is a low genetic diversity among them

At 88.5% similarity, the individuals of the 31 populations of Moroccan sesame were grouped into three large clusters and seven independent branches. Estimation of character associations could identify the relative importance of independent traits contributing to dependent ones and suggest upon the traits that may be useful as indicator for other traits. Genetic

differences among crop genotypes has become the primary need to grant patent and the protection of Plant Breeder Rights. ISSR marker was employed for the characterization of 16 sesame genotypes. ISSR primers were found effective in the discrimination of genotypes. A poor correlation was observed between the matrices produced ISSR primers, which might be due to the array of different sites of the genome.(Sharma, *et al.* 2009).

4.1.7 Genetic markers associated with yield

Seed yield in sesame like other field crops is a multifactorial character and direct selection for this trait may often be misleading. The components that determine the yield are best indices for selection. Therefore, knowledge of relationship between important yield traits and seed yield may help the researcher to identify suitable donors for a potential and successful breeding program (Kumaresan and Nadarajan 2002).

Several studies in recent years have found a high level of genetic variability for yield and yield related traits among sesame genotypes evaluated in different countries. The available genetic diversity in sesame germplasm is relatively limited as during sesame evolution many valuable genes associated with high yield might have been lost. In addition, Parameshwarappa, *et al.* (2010) analyzed the genetic divergence in 64 sesame genotypes for nine yield. Yield is a complex quantitative character controlled by many genes interacting with the environment and is the product of many factors called yield components. Selection of parents based on yield alone is often misleading. Hence, the knowledge about relationship between yield and its contributing characters is needed for an efficient selection strategy for the plant breeders to evolve an economic variety.

4.1.8 Genetic markers associated with drought tolerance

Abiotic stresses such as, drought, heat, salinity, and flooding threaten global food security. Crop genetic improvement with increased resilience to abiotic stresses is a critical component of crop breeding strategies. Most of the stress tolerance traits in wheat are polygenic and hence complex to understand at the physiological and molecular levels. Approaches like quantitative trait locus (QTL) mapping, marker assisted breeding, and introgression from wild gene pool are being employed to improve drought tolerance (Mwadzingeni, *et al.* 2016).

Drought stress is one of the consequences of climate change that has a negative impact on crop growth and yield. In the past, many simulation models were proposed to predict climate change and drought occurrences, and it is extremely important to improve essential crops to meet the challenges of drought stress which limits crop productivity and production.(Sallam, *et al.* 2019).

In the context of climate change and water scarcity, there is a need to develop and use drought-tolerant sesame cultivars, the significant effect of genotype, drought, and drought \times genotype interaction on all parameters investigated. Under severe drought, seeds of seven genotypes, including wild types, were not able to germinate. Kouighat *et al.* (2021) report that the sesame germplasm with such a high level of tolerance to drought during germination and early seedling growth stages Understanding the genetics behind drought stress tolerance as a quantitative trait influenced by genetic with many quantitative trait loci (QTLs) and environmental factors are remains a challenge for plant biologists and geneticists.

Genetic control of drought tolerance traits related requires intensive and integrative genetic, genomic and molecular researches to determine the genes underlying them and in which stage and mechanism or process they are involved.(Sallam, *et al.* 2019). By using gene association study, gene expression and transgenic experiments, a candidate gene *SiSAM* was identified to confer drought tolerance by modulating polyamine levels and ROS homeostasis in sesame (Dossa, *et al.* 2019).

4.2 Materials and Methods

4.2.1 Experimental Materials

4.2.1.1 Sample cultivation

Seeds of 60 sesames (*Sesamum indicium L.*) accessions were obtained from the Sudan National Gene Bank, Research Institute, Agricultural Plant Genetic Resources Conservation and Research Centre.

All the seeds accession genotypes samples were germinated in Petri dishes (90 mm diameter) containing Whatman filter paper soaked with 10 mL deionized water for four days.

4.2.1.2 Chemicals and equipment

The ISSR primers used in this study were of the following sequences (Table 4.1) PCR (Analytic Jena Germany 846-x- 070-301), Master Mixing PCR and DNA ladder (Solis bio dyne), Agarose (Condalab company), TBE buffer (Tris Boric acid EDTA) Fisher Bio Reagents, and DNeasy plant Mini Kit (QIAGEN GMBH 1,40724 Hilden Germany).

4.2.1.3 Primer Screening

A ten primers were used to determine the genetic diversity of the sesame genotypes. Polymerase Chain Reaction (PCR) was performed according to the annealing temperature of each primer.

4.2.2 Experimental methods

4.2.2.1 DNA extraction

Leaves samples of the 60 sesame genotypes were collected from five days The fresh leaf samples were ground with pestle and mortar. DNA extraction was

done according to the manual for DNA isolation from plant tissue using DNeasy Plant Maxi Kit (QIAGEN, Germany).

4.2.2.2 ISSR-PCR amplification

The polymerase chain reaction (PCR) was carried out by using automated thermal cycler (Analytic Jena Germany 846-x- 070-301). programmed in 20 μ l of mixture containing 4 μ l of Master Mixing PCR, 2 μ l of each of ISSR primers, 2 μ l of diluted DNA and 12 μ l of H₂O. The amplification was carried out as follows; 94°C for 5 min followed by 35 cycles of 1 min at 92°C, 1 min at 47°C, and 2 min at 72°C and 5 min at 72°C the reaction was finally stored at 16° C for ∞ .

4.2.2.3 Separation and photographing of PCR products

PCR products were separated on 1.5 % agarose gel in 1X TBE buffer (10.8 g Tris, 5.5g Boric acid, 4ml EDTA) per liter. Amplification product of each sample with 2 μ l loading dye were mixed and loaded on the gel. The length (number of base pairs) of each fragment was determined with reference to a 100 base pair DNA ladder. The electrophoresis was done for 35 minutes at constant voltage of 100v.

To allow visualization of bands, agarose gel was stained with ethidium bromide. Finally, gel picture was taken and visualized under UV light using UV-trans illuminator (analytic Jenaus CA 9186 USA). The bands were photographed with digital camera for further analysis.

4.2.3 Molecular Scoring and data analysis

Each amplification product position was considered a particular locus. The amplified products for each primer at a specific position in a gel were scored

visually for each ISSR marker as “1” for present and “0” for absent to generate a binary data matrix. The length (base pair number) of each fragment in the amplified product was determined with reference to a 100 DNA base were excluded from analysis Scoring and data analysis: -

Amplified bands AB

Polymorphic bands PB

Percent of polymorphism P %

Fragment size and base pair FS and bp

Calculate pic polymorphism information content

$$Pic = 1 - (p^2 + q^2)$$

P ≡ frequency of the allele band present

q ≡ frequency of the allele band absent

Calculate the marker index (MI) for each ISSR as

$$MI = pic \times \eta\beta$$

Pic ≡ value

η ≡ no. of band

β ≡ proportion of polymorphic band

Table 4.1: ISSR primer sequence

Primer code	Sequence
SR-02	ACACACACACACACACG
SR-05	ACACACACACACACACYC
SR-09	AGAGAGAGAGAGAGAGC
SR-13	AGAGAGAGAGAGAGAGYC
SR-15	AGAGAGAGAGAGAGAGYT
SR-18	ATGATGATGATGATG
SR-19	ATGATGATGATGATGATG
SR-20	CACACACACACACACAG
SR-23	CTCTCTCTCTCTCTRG
SR-25	CTCCTCCTCCTCCTT

4.3 Result and discussion

4.3.1 Banding pattern and polymorphism

Table 4.2 showed the polymorphic information content (PIC) and marker indexes of ISSR primers used in this study. The DNA amplification pattern of the ISSR primers was reproducible with 1.5% agarose gel electrophoresis. Out of the 12 primers initially tested, 10 of them gave relatively clear banding pattern based on the results from 60 DNA samples ten ISSR primers were similar and all of them produced 107 bands. Shown similar polymorphic bands. The size of the amplified products ranged between 3000-200 bp.

The genetic characters of the 60 sesame genotypes which characterized with the 10 primers showed that all the primer were completely identical shown a level of 100% polymorphism.

In ISSR analysis number of amplified products (bands) obtained was specific to each primer. The polymorphic information content (PIC)% for the evaluated primers vary considerably and ranged between 0.362593(Primer 23) and 0.214444 (primer 20).

These results disagree with (Singh, *et al.* 2015). They said that ISSR analyses, a total of 183 alleles were detected among which 164 alleles (89.61%) were polymorphic. The number of alleles per primer ranged from 2 to 15 with an average of 7.32 alleles per ISSR primer. In addition, 12 selected SSR primers generated 41 amplified bands. The fragment size varied from 155 to 371 bp.

Ten ISSR primers sequences (Table 4.2) were previously reported by Reddy et al (2002), Sharma et al (2009), Abate et al (2015) as a high polymorphism producer of all sesame genotypes, which were utilized to identify the level of

genetic variability for sesame genotypes under investigation in relation to geographical origins of sesame.

On the other hand, (Sharma, *et al.* 2009). Reported that 17 ISSR primers that generated clear and reproducible banding patterns amplified 163 bands, respectively among 16 sesame genotypes. Though, there was greater similarity among sesame genotypes (0.71 for ISSR), the observed genetic diversity (0.29 for ISSR), was found effective for the characterization of sesame genotypes.

A total of 57 bands were obtained with ISSR primers, of which 47 were polymorphic. The PIC ranged from 0.002 to 0.350, showing that ISSR markers are informative and relevant the populations evaluate. for discriminating The results obtained showed that Moroccan sesame populations are characterized by a low genetic diversity, suggesting a genetic proximity among them. Therefore, new germplasm should be either introduced from diverse geographical origins or created through mutagenesis breeding in order to broaden the existing genetic variability (Meriem, *et al.* 2021).

Anggraeni, and Fadilah, (2022) determined the genetic relationship between 81 accessions of sesame plants using ISSR markers. The analysis produced 29 DNA bands which 100% are polymorphic bands. ISSR markers can identify sesame germplasm diversity and provide helpful information for sesame breeding and germplasm management programs. However, Sharma, *et al.* (2009) similarly results. Molecular characterization revealed a polymorphism percentage of 46.66% for ISSR markers, the cluster analysis showed a high genetic diversity among the sesame genotypes and their diversity was consistent with their source pedigrees. Considering the

relatedness of genotypes, geographical origin and their morphological characteristics reflected to the similarity of ISSR patterns (Markers, 2020).

The 7 ISSR primers in 128 sesame genotypes yielded 96 reproducible amplified bands. The number of amplified bands varied from 7 to 19. Out of 96 bands, 89 (92.2%) were polymorphic. Average number of bands and polymorphic bands per primer were 14 and 12.6 respectively. The polymorphic information content (PIC) value ranged between 0.26 and 0.76, showing the high informativeness of the selected primers. (Abate, *et al.* 2015).

Kumar and Sharma (2011) to determine the genetic diversity and relationships among 20 commercially cultivated sesame genotypes representing different geographical regions. A total 279 band positions with 57% polymorphism was amplified by ISSR primers. The ISSR primers with high discrimination power were identified producing genotype-specific bands and distinguishing all the sesame genotypes.

Table 4.2 : Polymorphic information content and Marker indexes of ISSR primers used in this study

Primer No.	Amplified bands	Polymorphic bands	Percent of polymorphic bands	Fragment sizes	Polymorphic Information Content (PIC%)	Marker Index
SR-02	13	13	100	1500 to 300bp	0.273034	3.549444
SR-05	12	12	100	1800 to 200	0.297731	3.572778
SR-09	7	7	100	1000 to 400	0.22754	1.592778
SR-13	15	15	100	3000to200	0.316259	4.743889
SR-15	12	12	100	2000to200	0.24625	2.955
SR-18	9	9	100	1300to300	0.344568	3.101111
SR-19	9	9	100	1800to400	0.312222	2.81
SR-20	9	9	100	1000 to 200	0.214444	1.93
SR-23	12	12	100	2000 to 300	0.362593	4.351111
SR-25	9	9	100	2000 to 400	0.257037	2.313333

4.3.2 Clustering Analysis and Relationships among Sesame Genotypes

UPGMA analyses were used to construct a dendrogram for the 60 genotypes of sesame Sesame cultivars based on the 107 bands obtained from the 10 primers. were divided into two major groups (I, II) Depend on geographical distribution (west and east of Sudan) respectively, group (I) was subgrouped(A&B) into 4 clusters depend on the area west Sudan which divided in to Darfur and kordfan (Fig. 4.1).

Genotypes of sesame landraces were widely distributed and formed separate clusters. The most distinct genotypes were accession No. 44 (Fig. 4.1). Genetic distances among the landraces were larger than distances between the released cultivars.

In general, there was no clear clustering in the UPGMA tree. The majority of the landraces from different geographical regions were inter-mixed and grouped together in the same clusters with the exception of some genotypes, which were found to group from any of the population such as, Blue Nile, these change Cluster analyses of the present study showed that most genotypes of the landraces did not group together with other genotypes from the same geographical region. The close relationship between some sesame genotypes from the different populations might be due to gene flow caused by the exchange of germplasm through farmers and traders across regions of Sudan.

Anggraeni, and Fadilah, (2022) found that phylogenetic analysis, the sesame collections clustered into seven major groups. There was clear separation of some accessions based on their geographical origin. ISSR markers can

identify sesame germplasm diversity and provide helpful information for sesame breeding and germplasm management programs.

Abate et al., (2015) reported that genetic associations among individual genotypes were determined based on dissimilarity matrix using Darwin version 5.0 and a Neighbor-Joining hierarchical tree was constructed based on UPGMA.

Similar results were obtained for the (Singh, et al. 2015) Dendrogram based on UPGMA analysis separated nearly all the genotypes clearly into their respective groups. Genotypes from Uttar were clustered into a single group which further separated into two subgroups according to their state of origin.

A poor correlation and lack of perfect congruence between phenotypes and marker-based analysis was indicated by the UPGMA dendrogram and PCA analysis. Kumar and Sharma (2011) We recommend the use of molecular markers for testing, required for obtaining intellectual property rights, and also for the analysis of genotypic diversity among sesame varieties.

4.3.3 ISSR Regression

The poor correlation between stresses and molecular marker in all accession of sesame based analysis was indicated by explain Regression Test R Square less than 0.5.

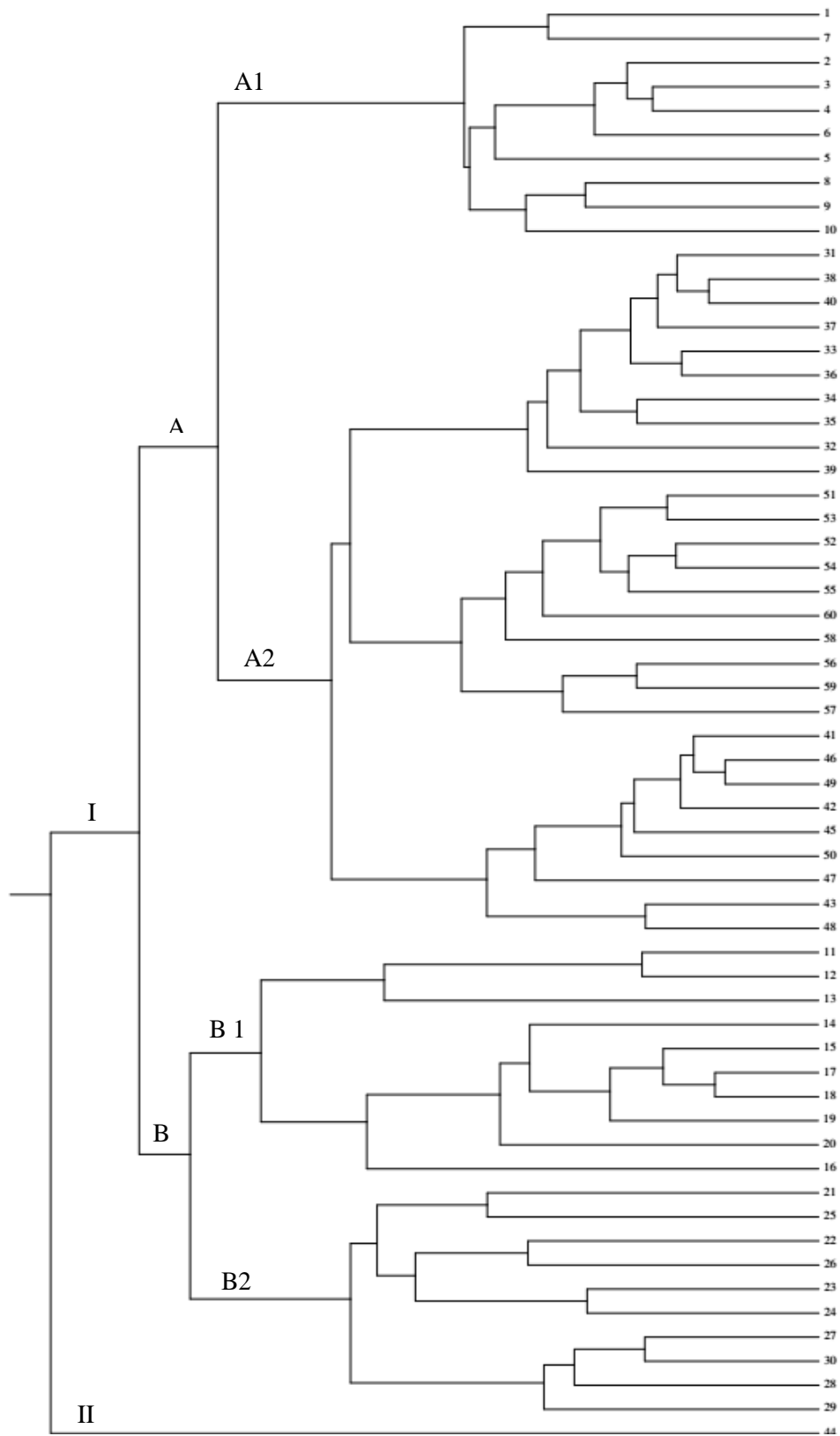


Figure 4.1 UPGMA clustering dendrogram of *Sesamum indicum* cultivars based on average genetic distance by using ISSR

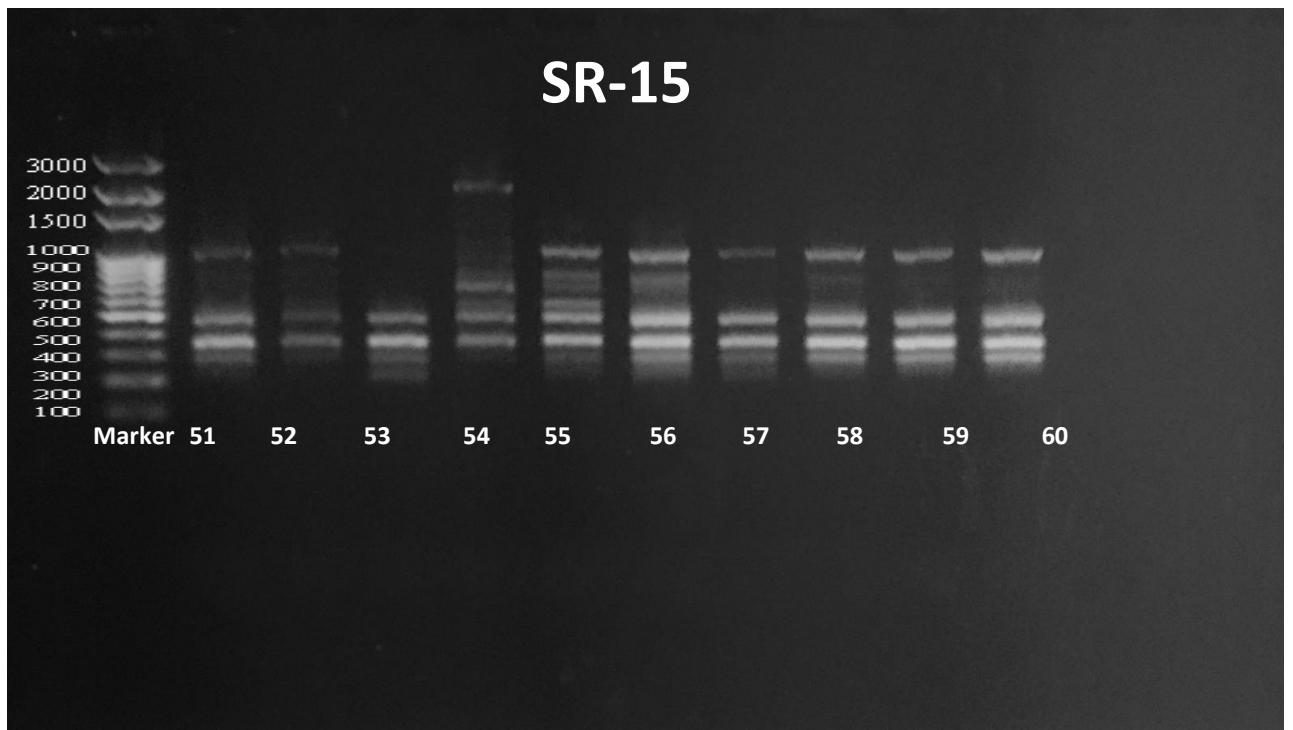


Figure 4.2 Banding patterns of ISSR primer 15 for 10 sesame cultivars

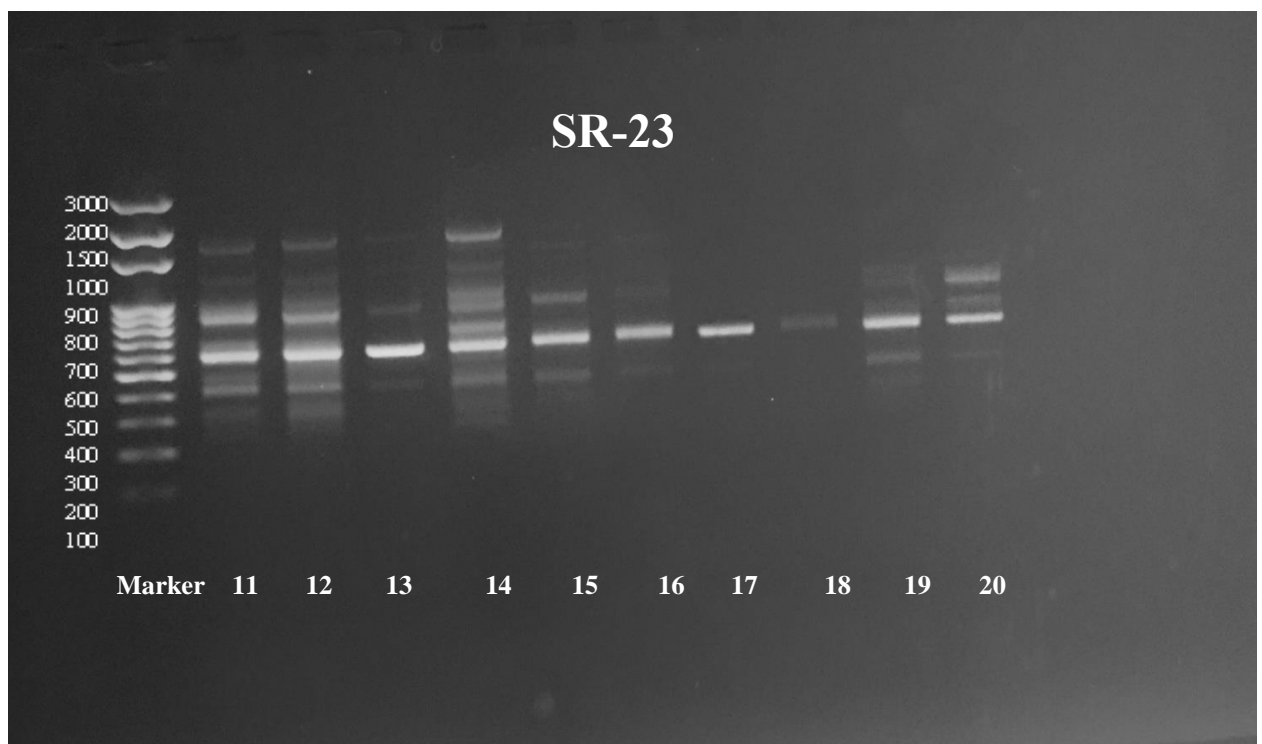


Figure 4.3 Banding patterns of ISSR primer 23 for 10 sesame cultivars

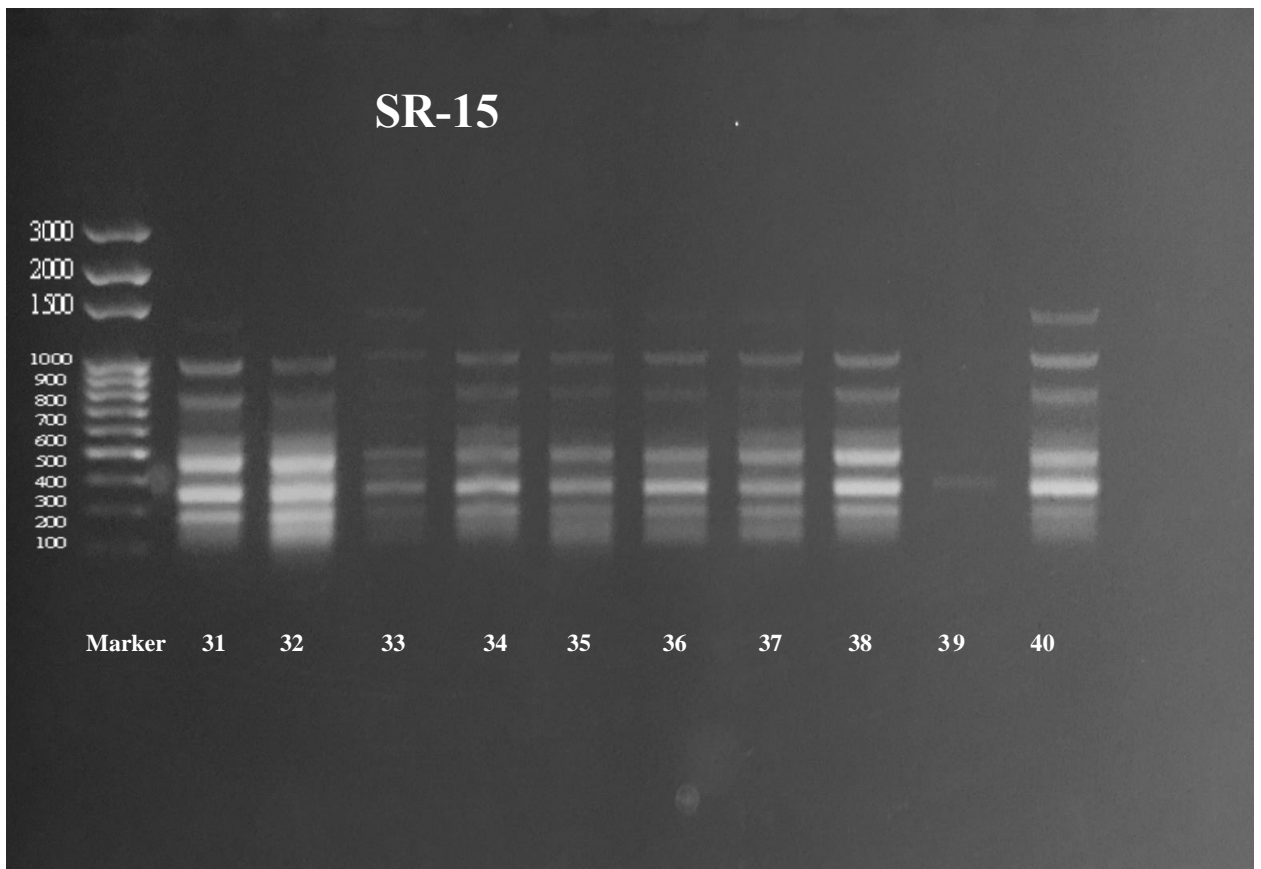


Figure 4.4 Banding patterns of ISSR primer 15 for 10 sesame cultivars

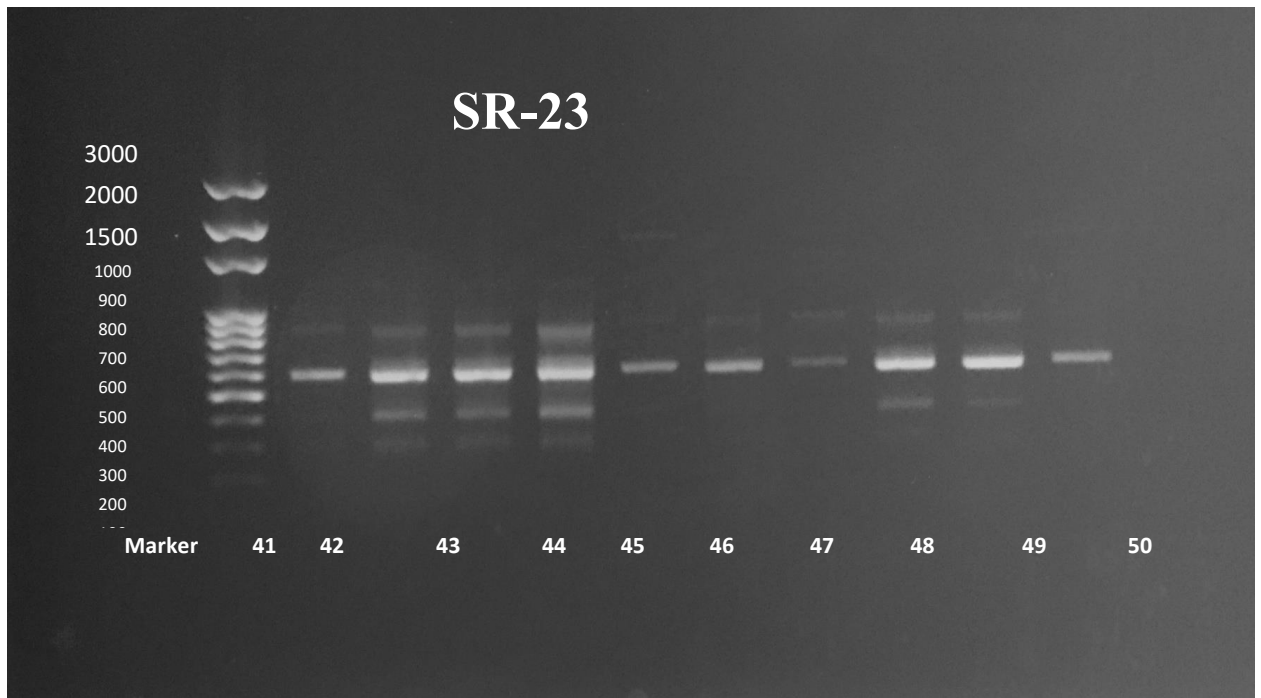


Figure 4.5 Banding patterns of ISSR primer 23 for 10 sesame cultivars

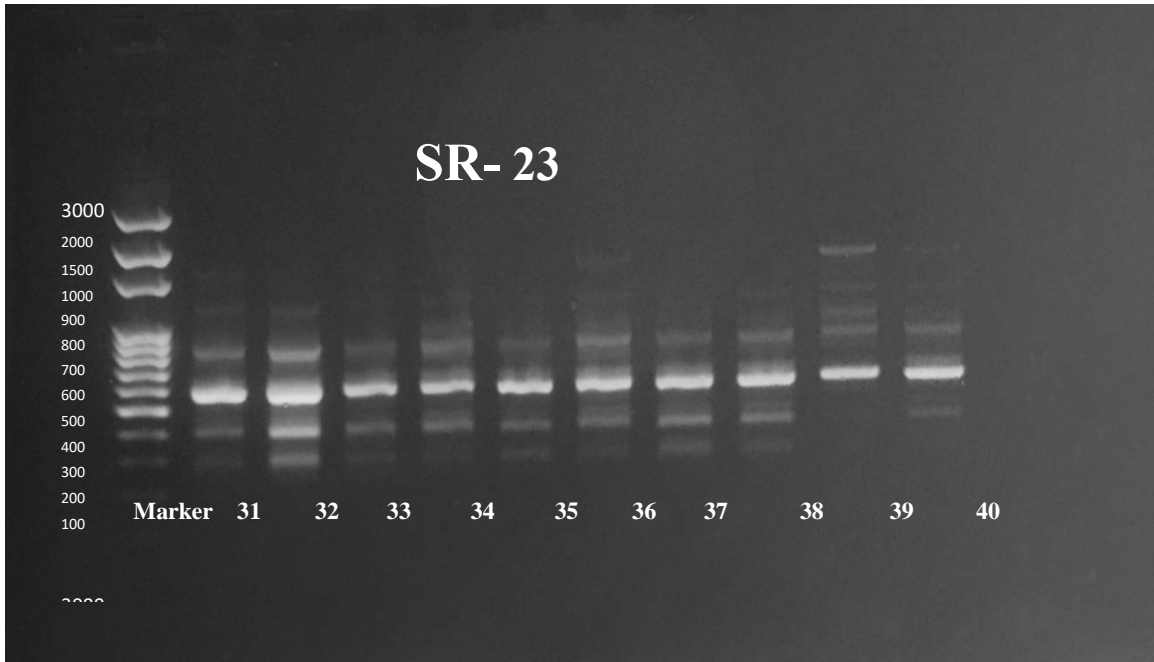


Figure 4.6 Banding patterns of ISSR primer 23 for 10 sesame cultivars

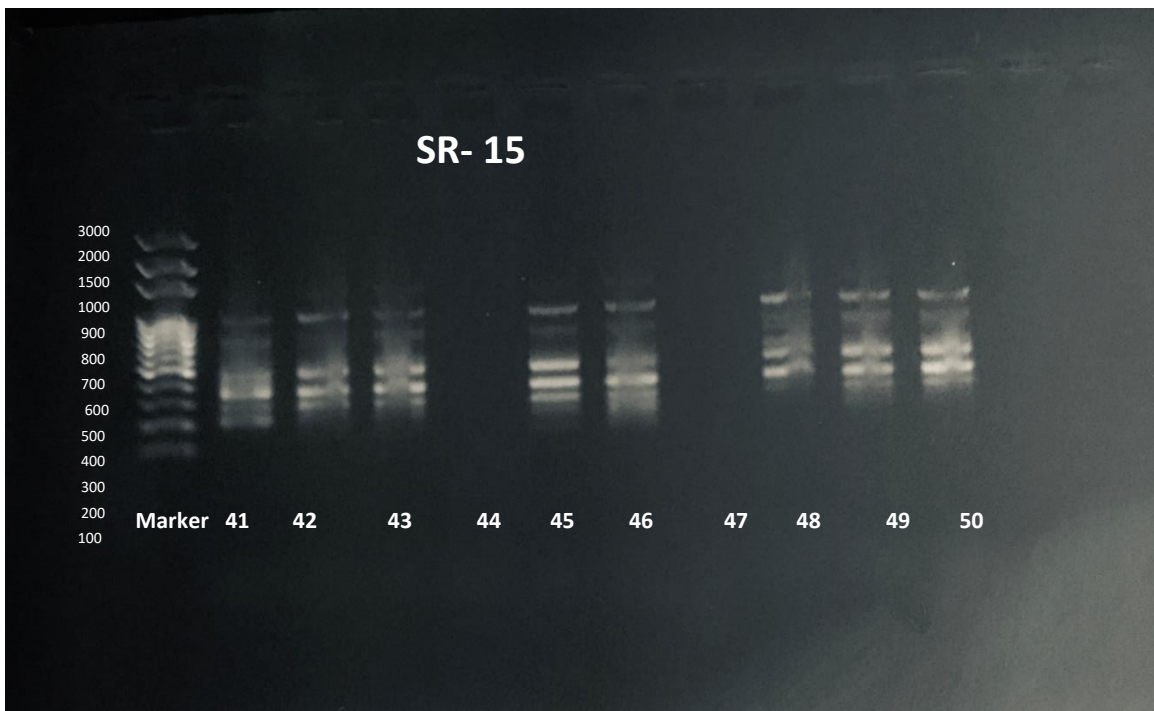


Figure 4.7 Banding patterns of ISSR primer 15 for 10 sesame cultivars

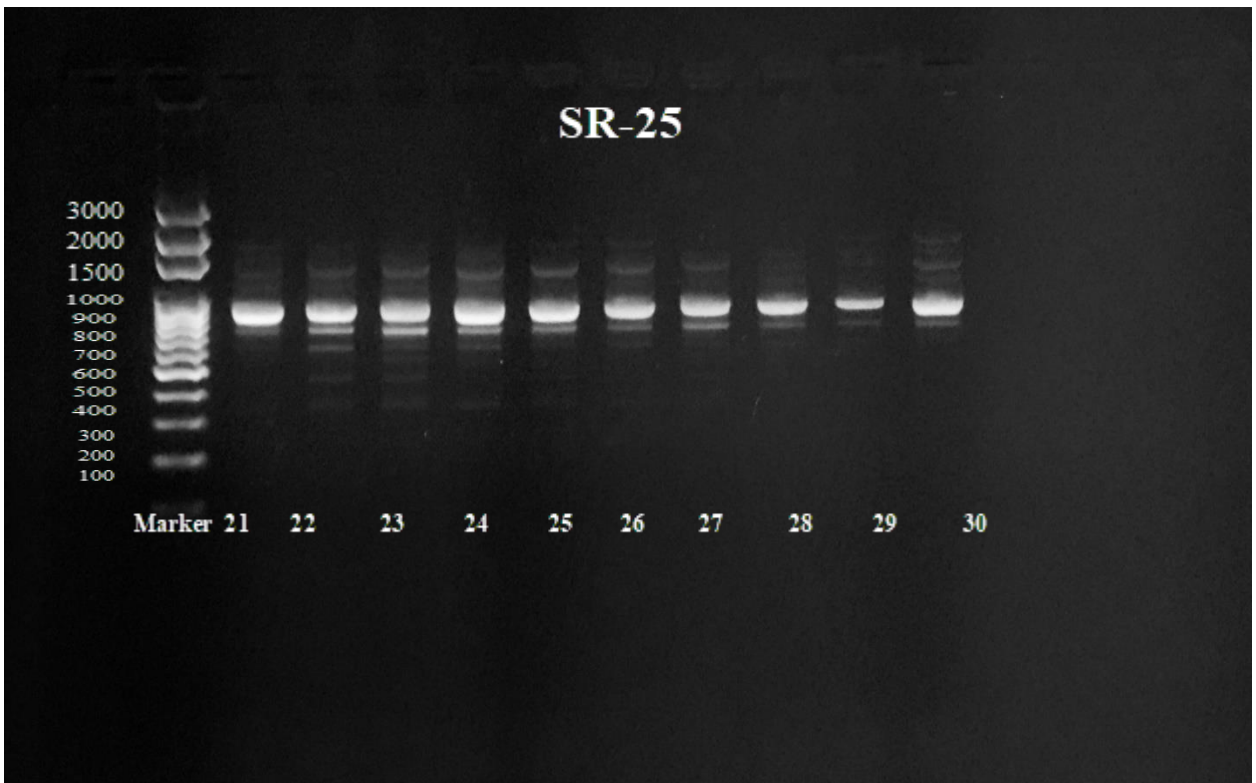


Figure 4.8 Banding patterns of ISSR primer 25 for 10 sesame cultivars

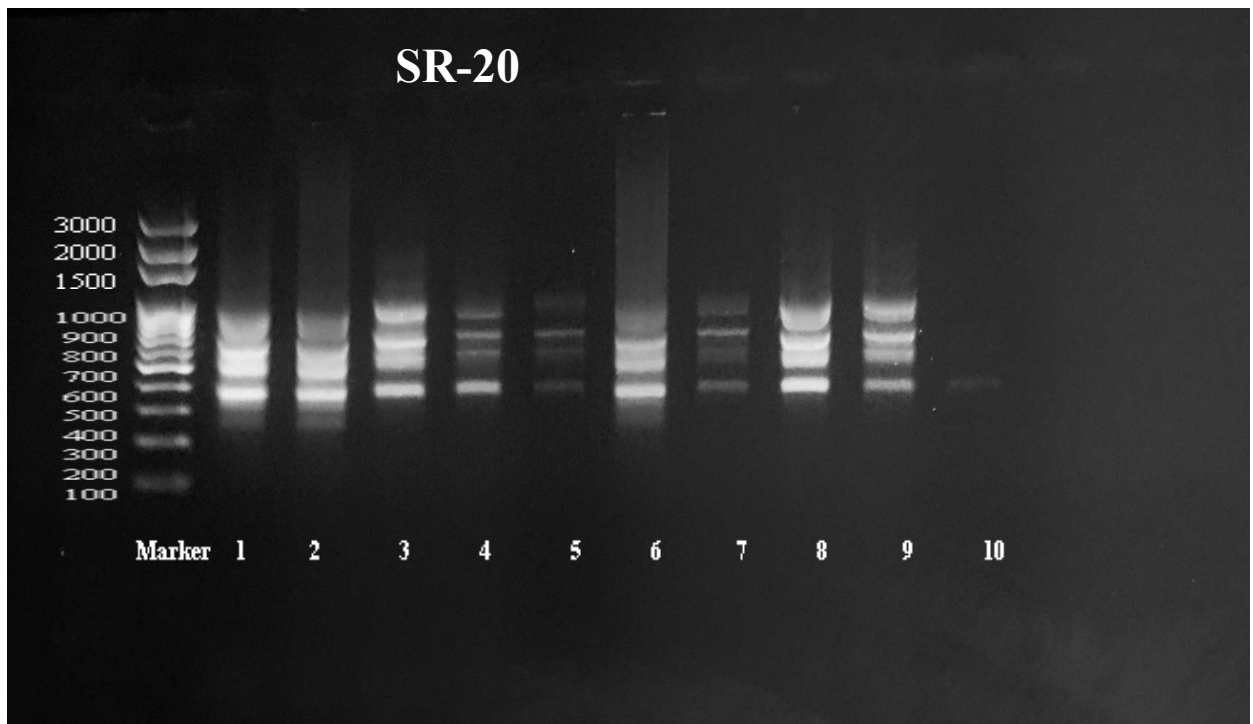


Figure 4.5 Banding patterns of ISSR primer 20 for 10 sesame cultivars

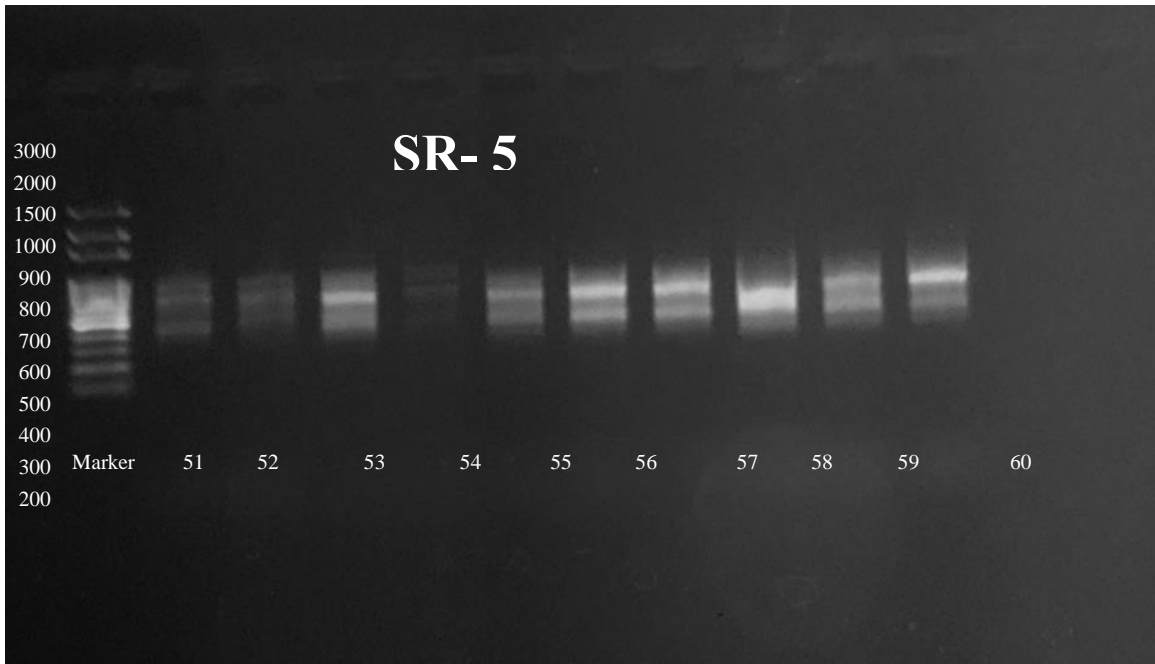


Figure 4.7 Banding patterns of ISSR primer 20 for 10 sesame cultivars

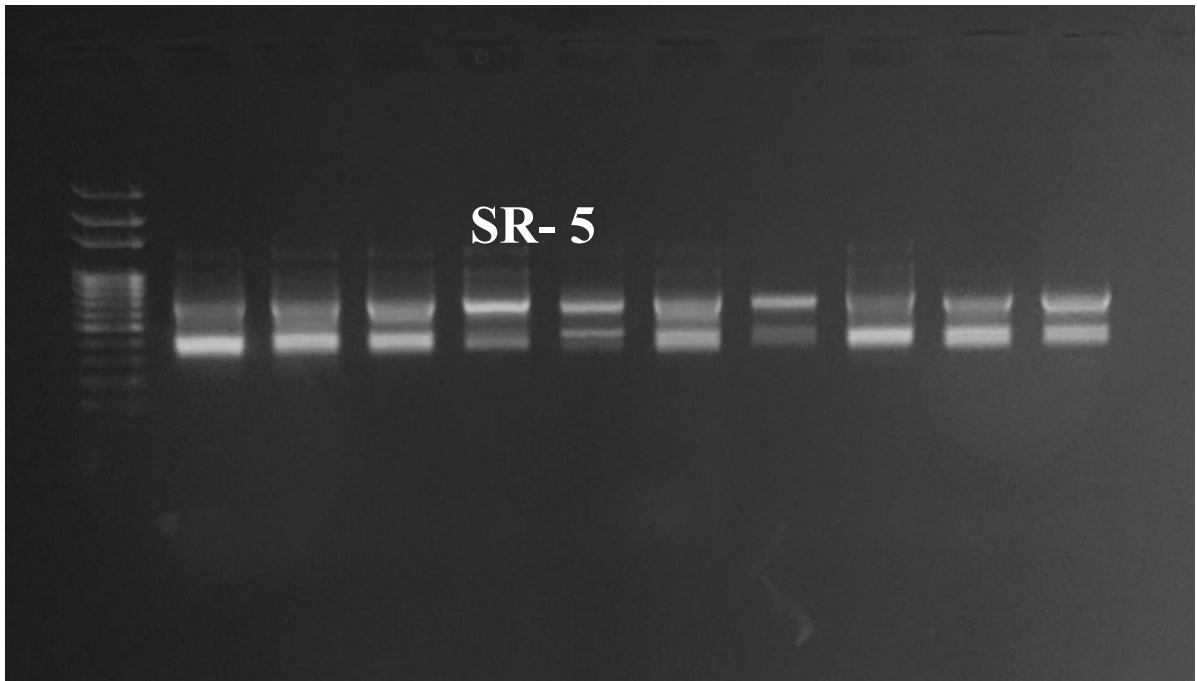


Figure 4.6 Banding patterns of ISSR primer 5 for 10 sesame cultivars

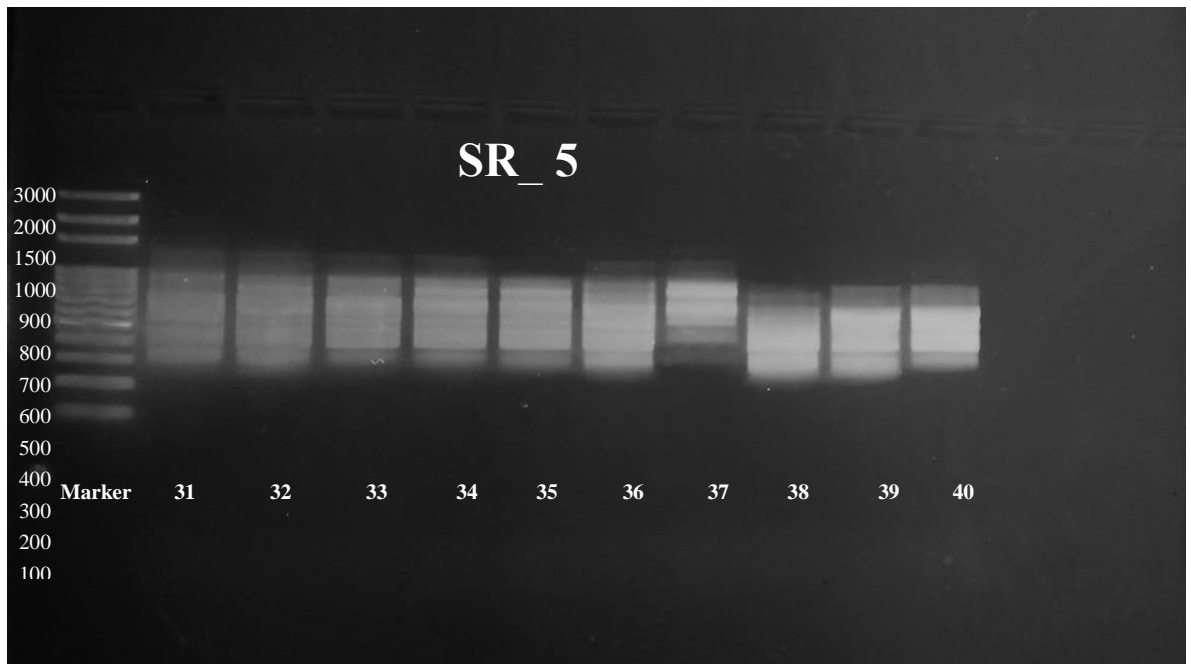


Figure 4.8 Banding patterns of ISSR primer 5 for 10 sesame cultivars

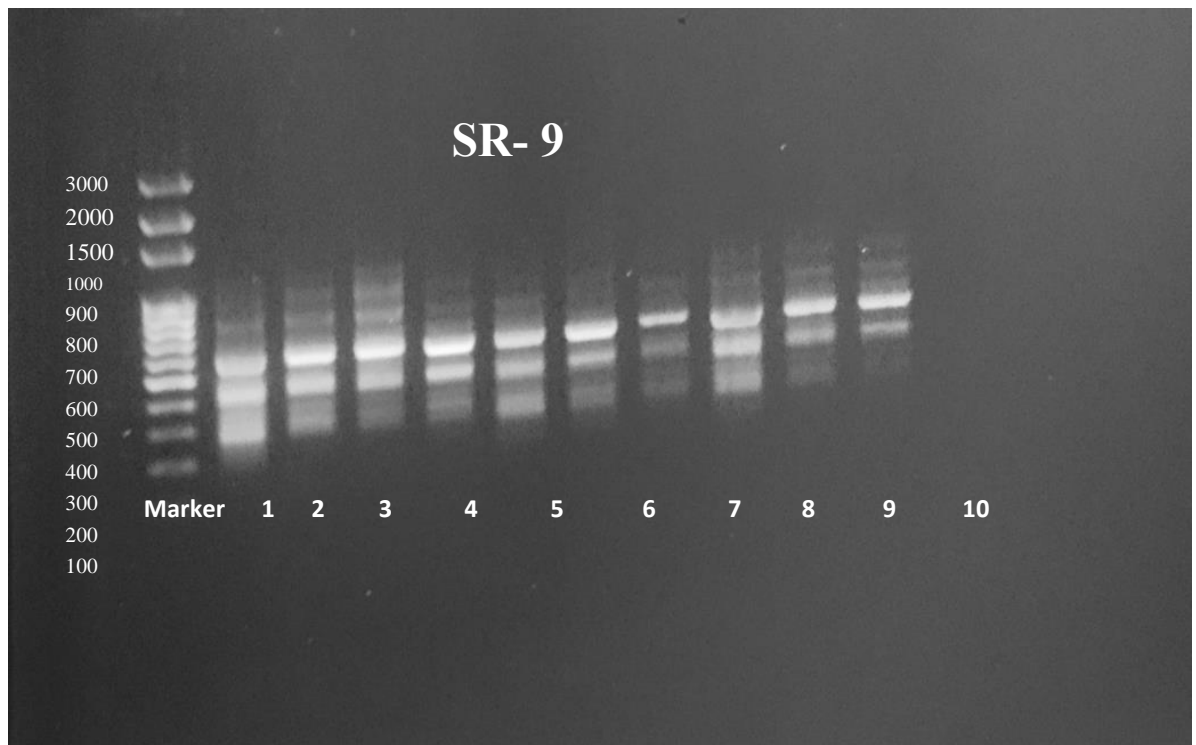


Figure 4.9 Banding patterns of ISSR primer 9 for 10 sesame cultivars

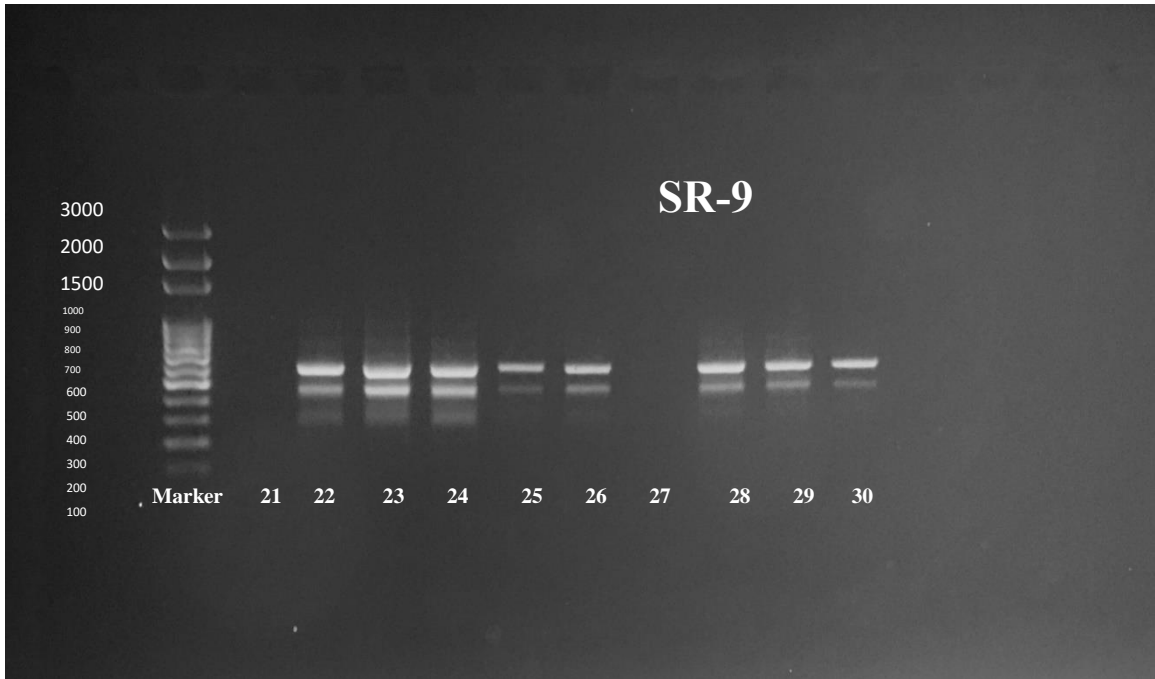


Figure 4.11 Banding patterns of ISSR primer 9 for 10 sesame cultivars

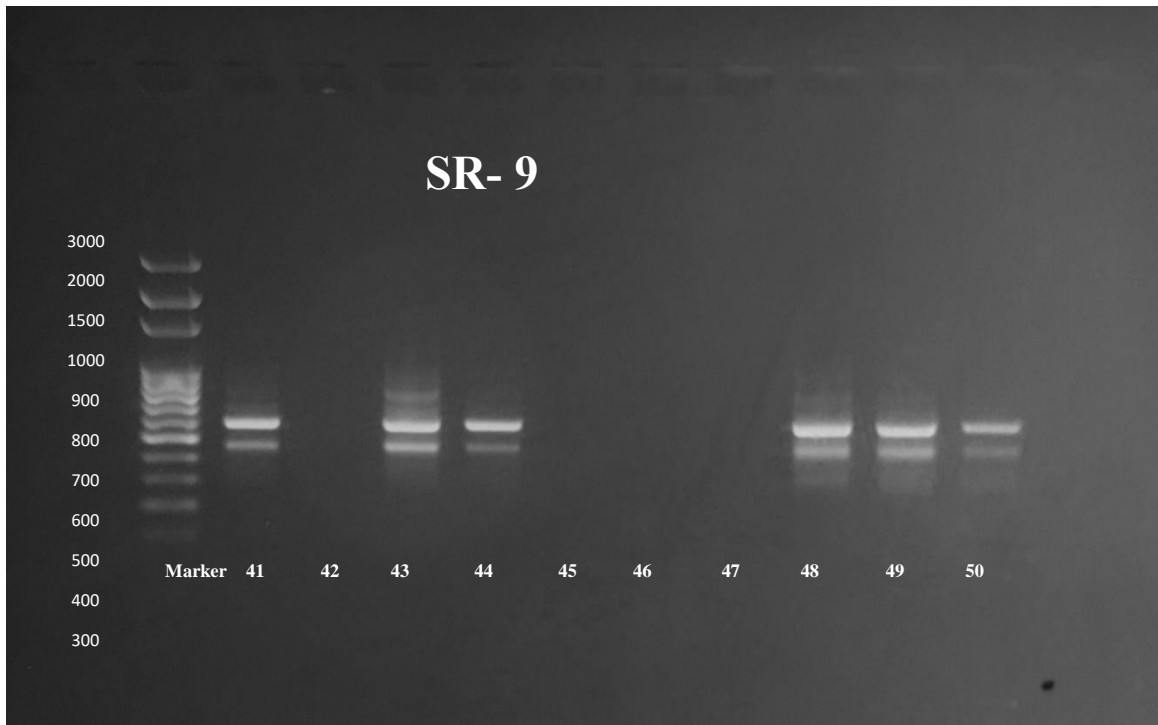


Figure 4.10 Banding patterns of ISSR primer 9 for 10 sesame cultivars

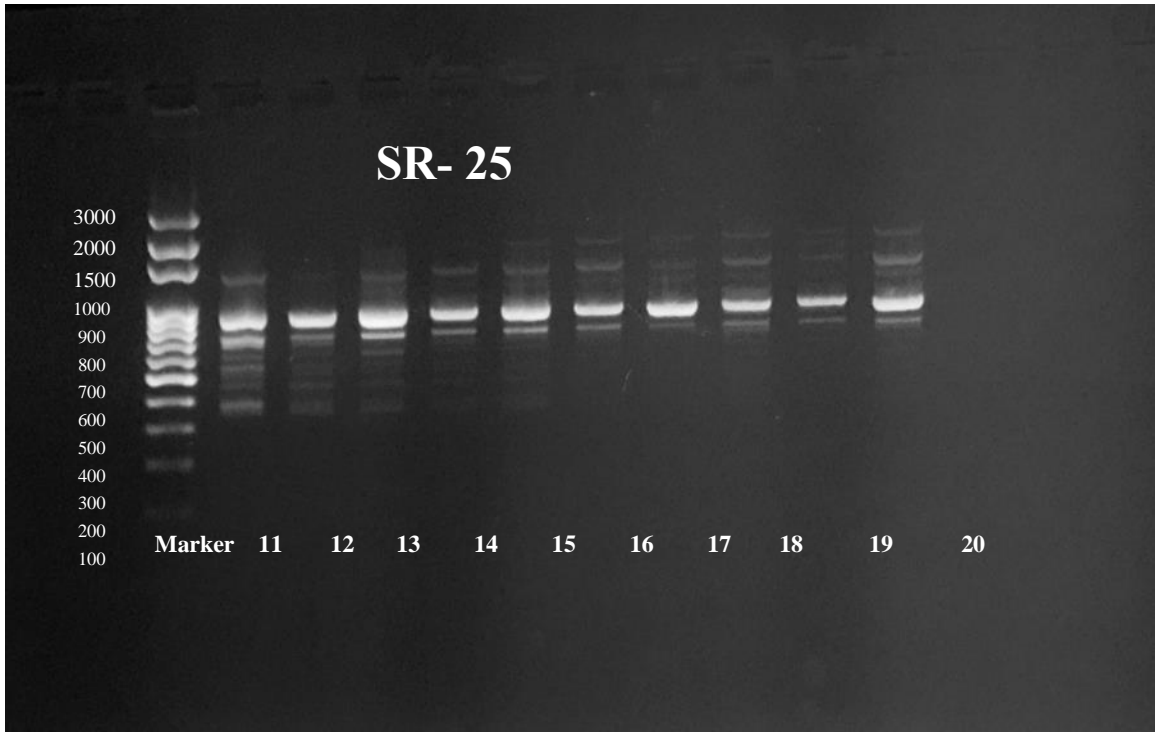


Figure 4.12 Banding patterns of ISSR primer 25 for 10 sesame cultivars

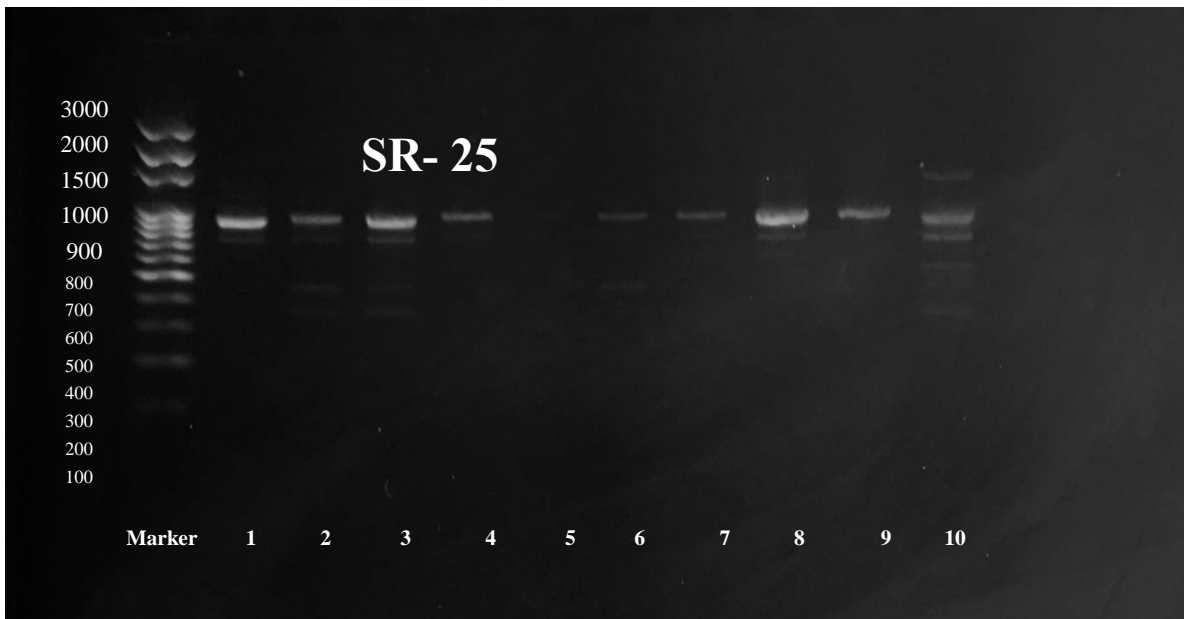


Figure 4.13 Banding patterns of ISSR primer 25 for 10 sesame cultivars



Figure 4.14 Banding patterns of ISSR primer 19 for 10 sesame cultivars

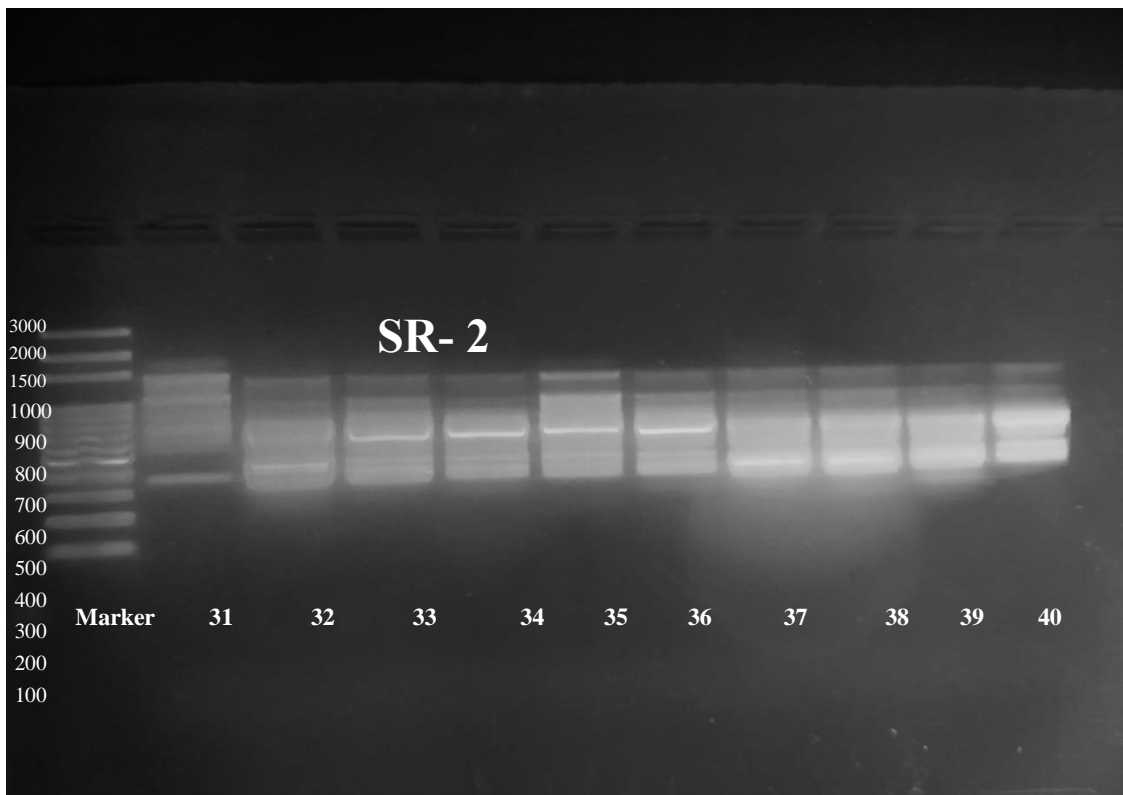


Figure 4.19 Banding patterns of ISSR primer 19 for 10 sesame cultivars

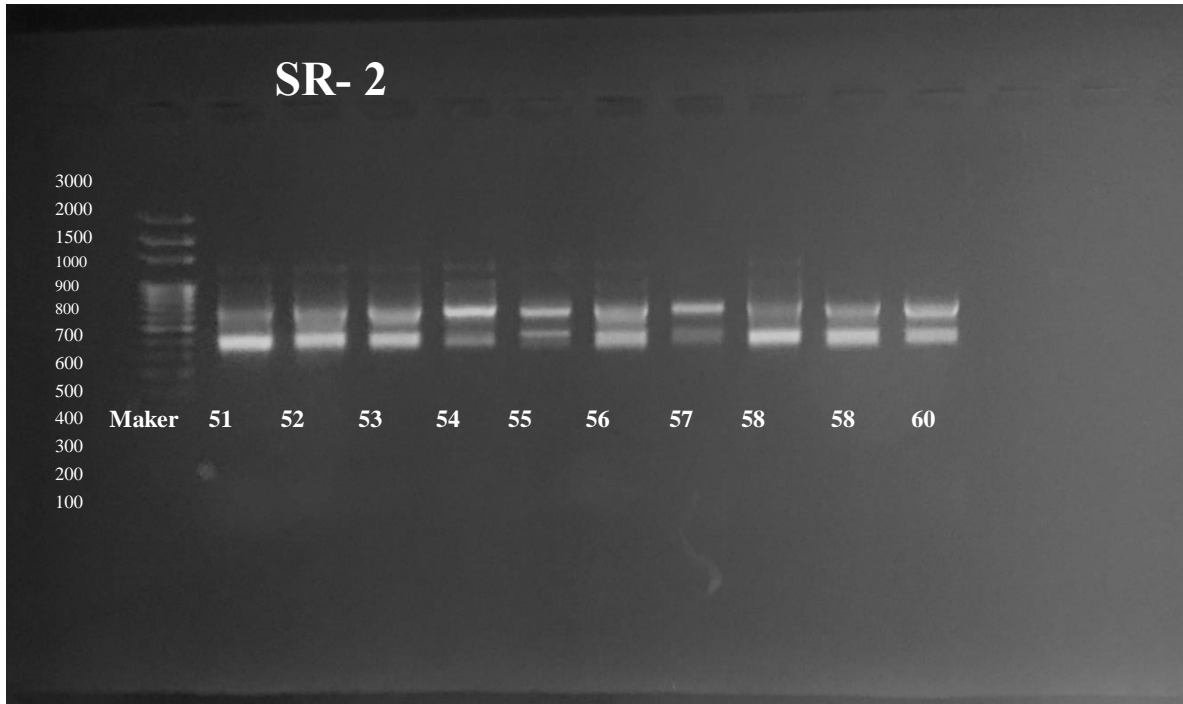


Figure 4.15 Banding patterns of ISSR primer 2 for 10 sesame cultivars

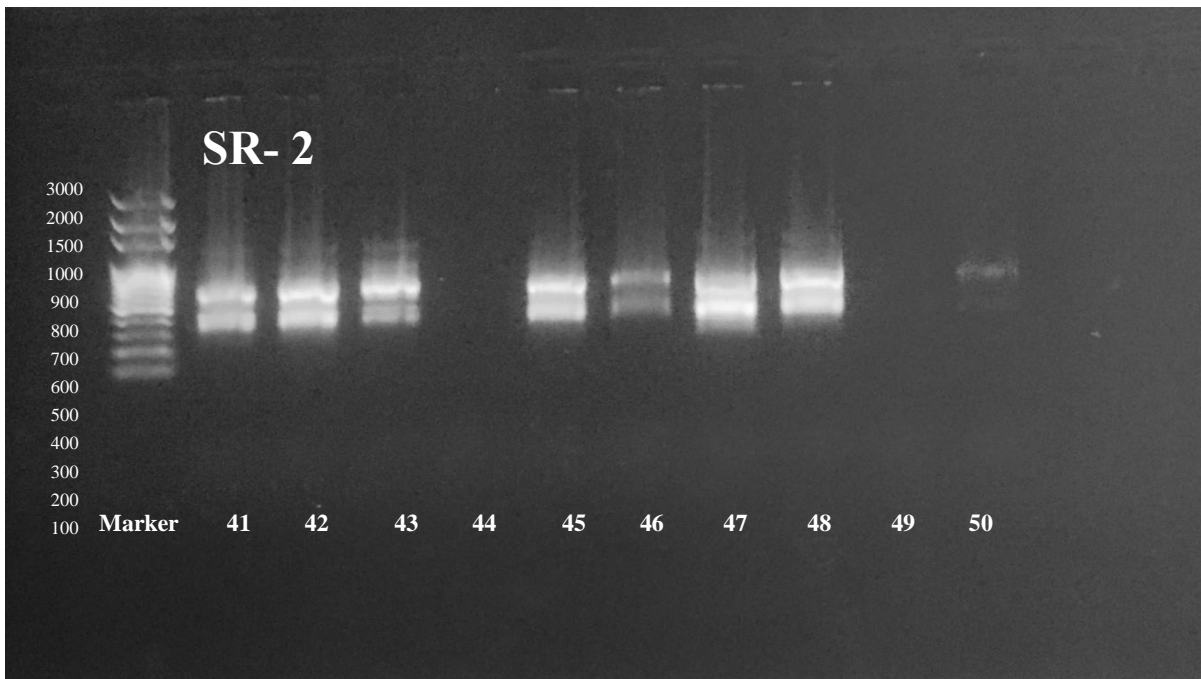


Figure 4.16 Banding patterns of ISSR primer 2 for 10 sesame cultivars

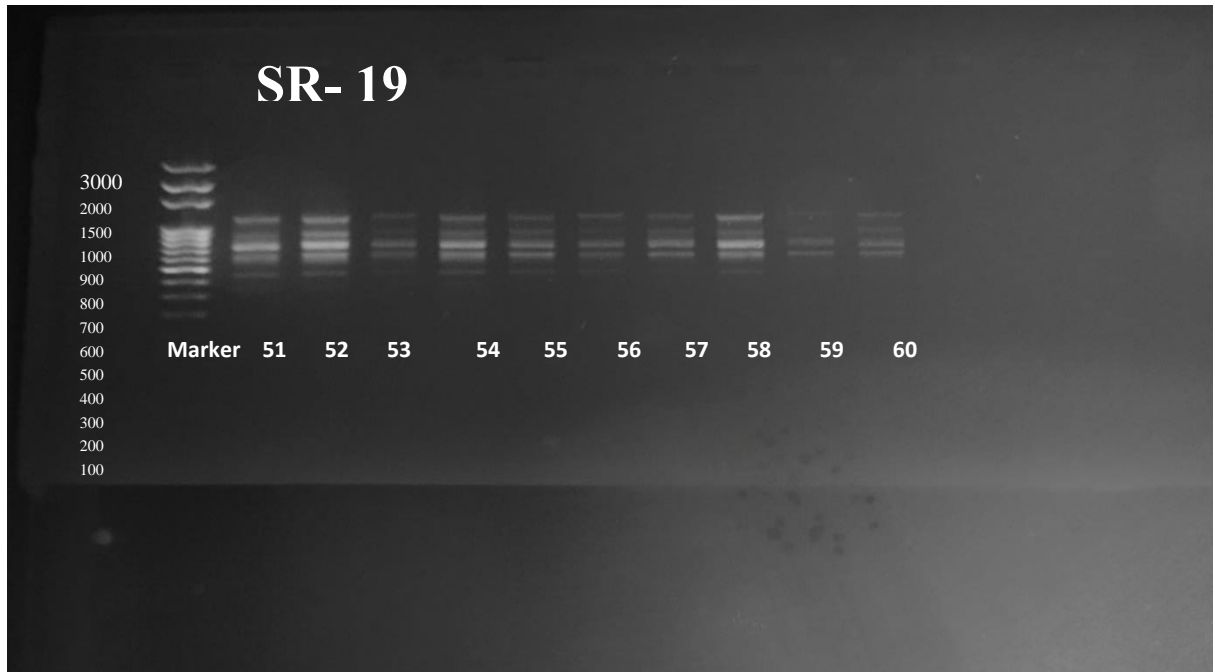
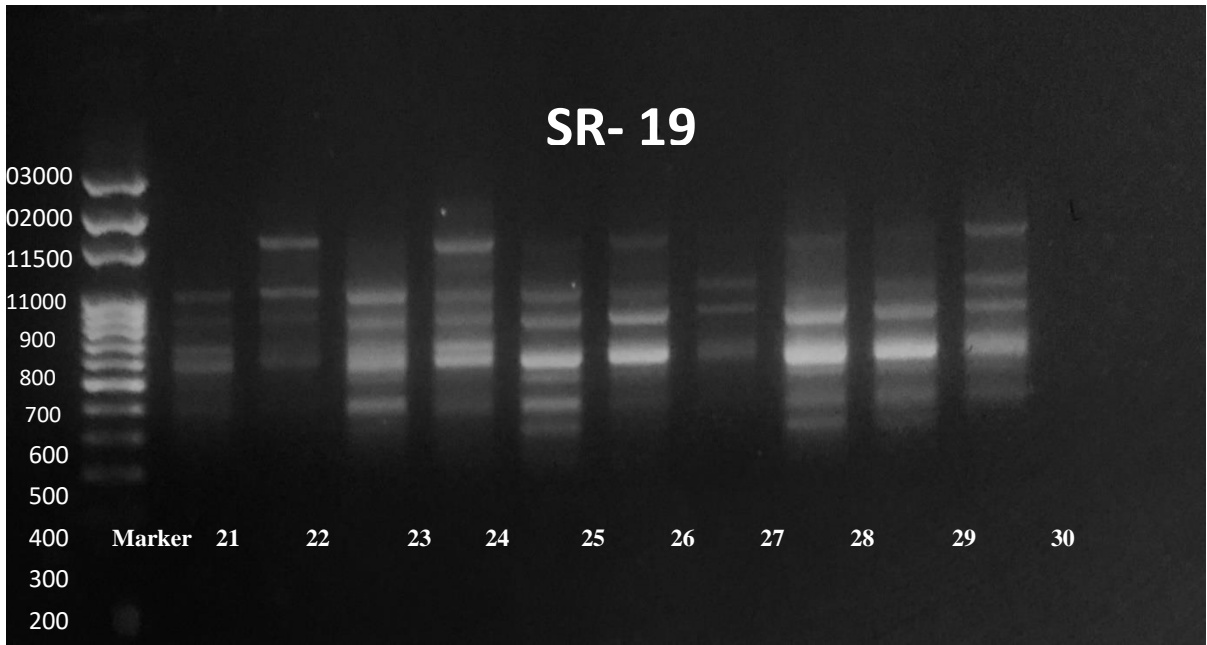


Figure 4.18 Banding patterns of ISSR primer 19 for 10 sesame cultivars

Figure 4.17 Banding patterns of ISSR primer 19 for 10 sesame cultivars

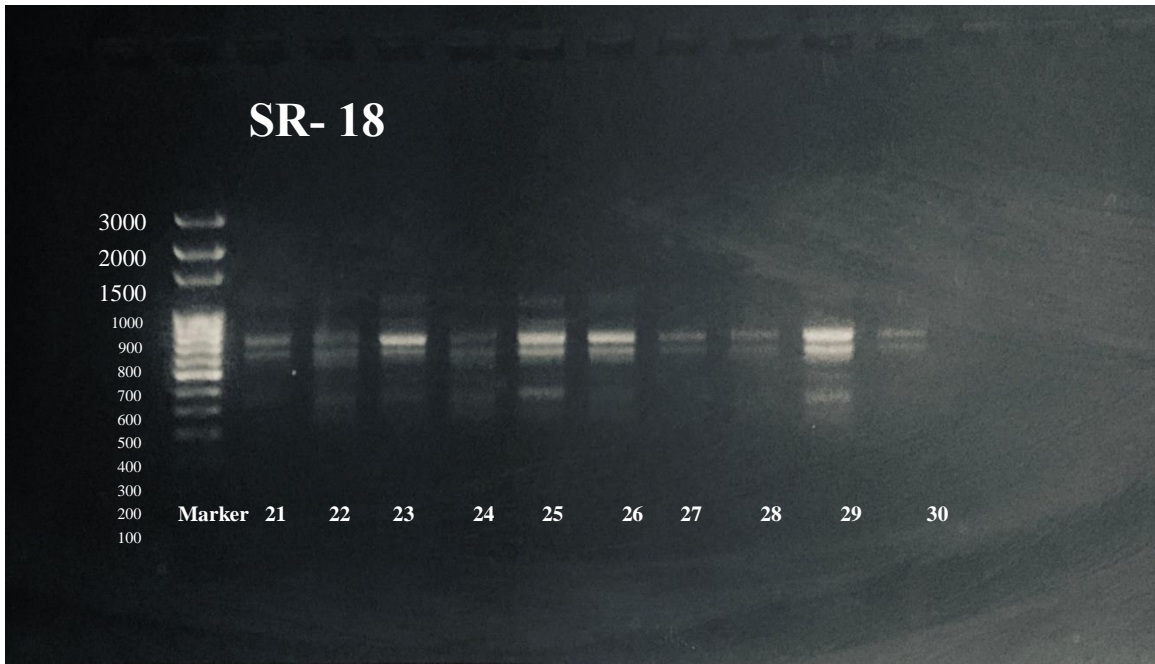


Figure 4.19 Banding patterns of ISSR primer 18 for 10 sesame cultivars

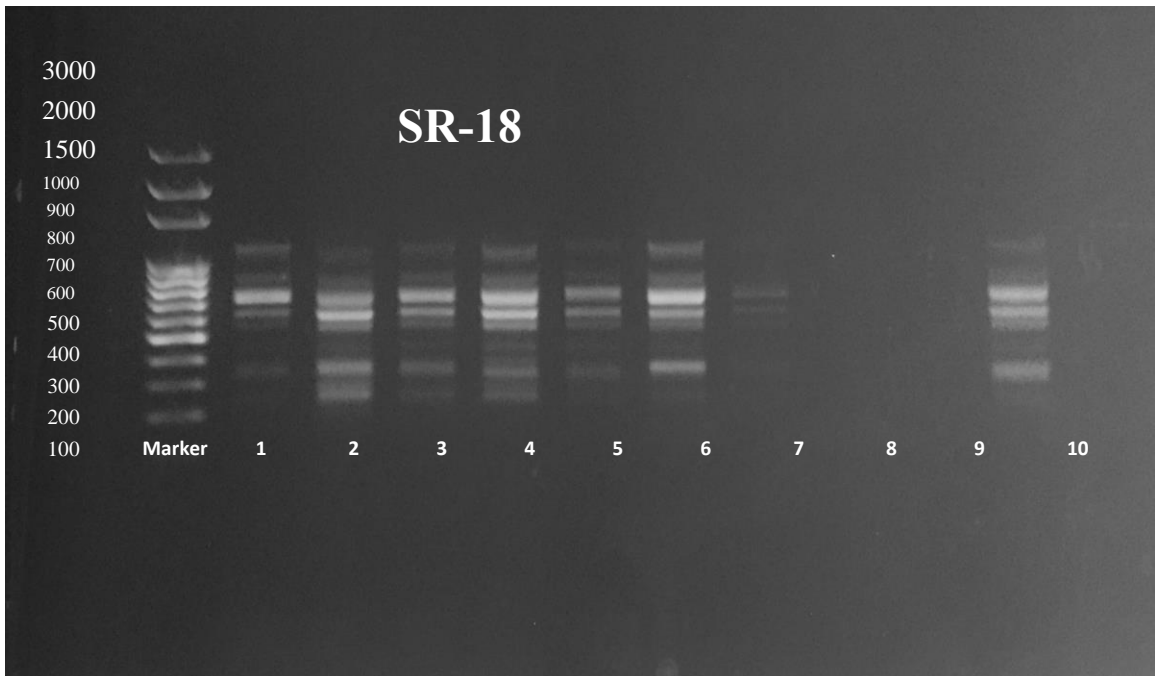


Figure 4.20 Banding patterns of ISSR primer 18 for 10 sesame cultivars

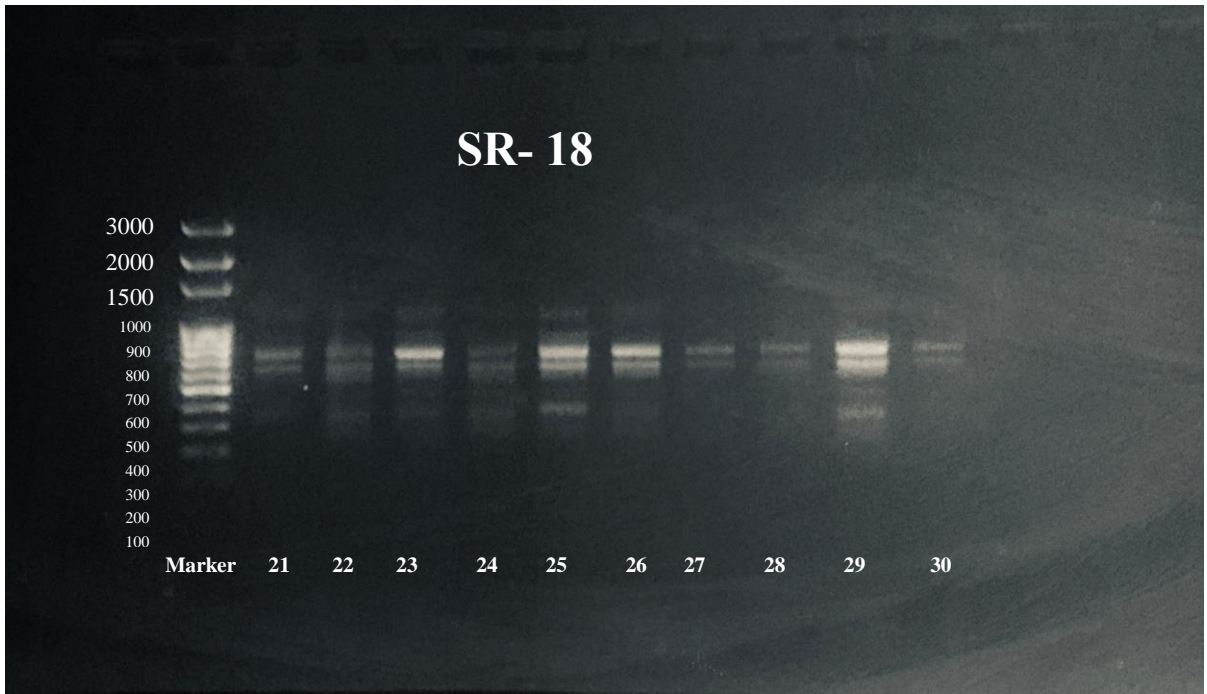


Figure 4.21 Banding patterns of ISSR primer 18 for 10 sesame cultivars

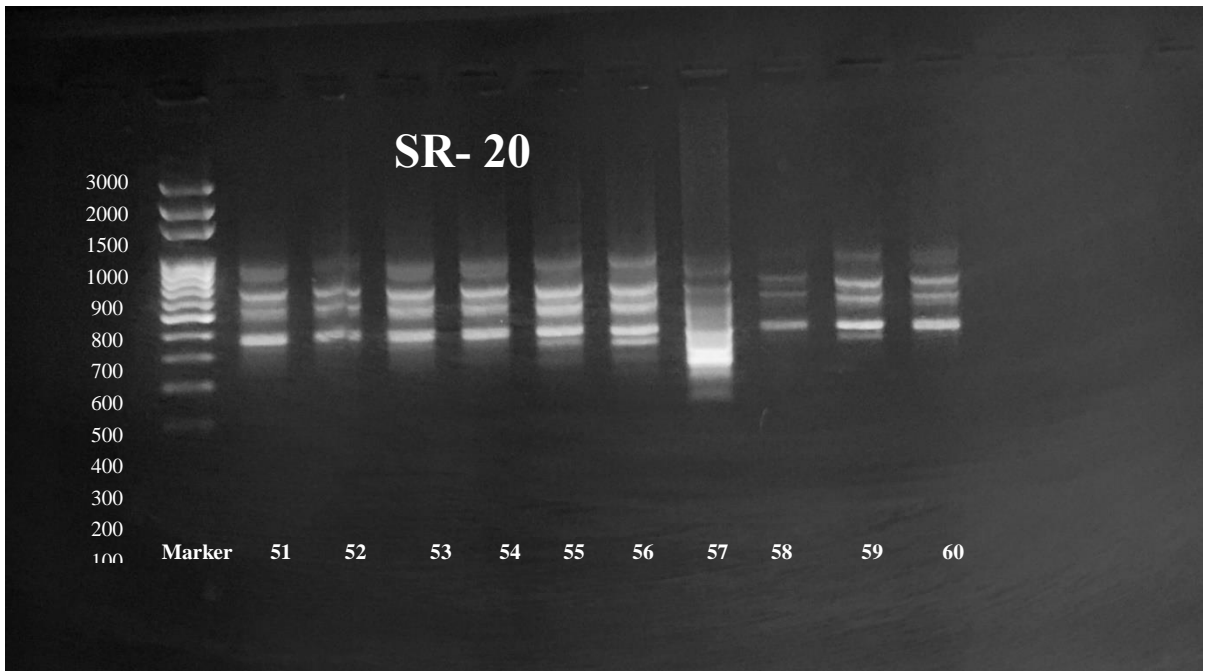


Figure 4.29 Banding patterns of ISSR primer 20 for 10 sesame cultivars

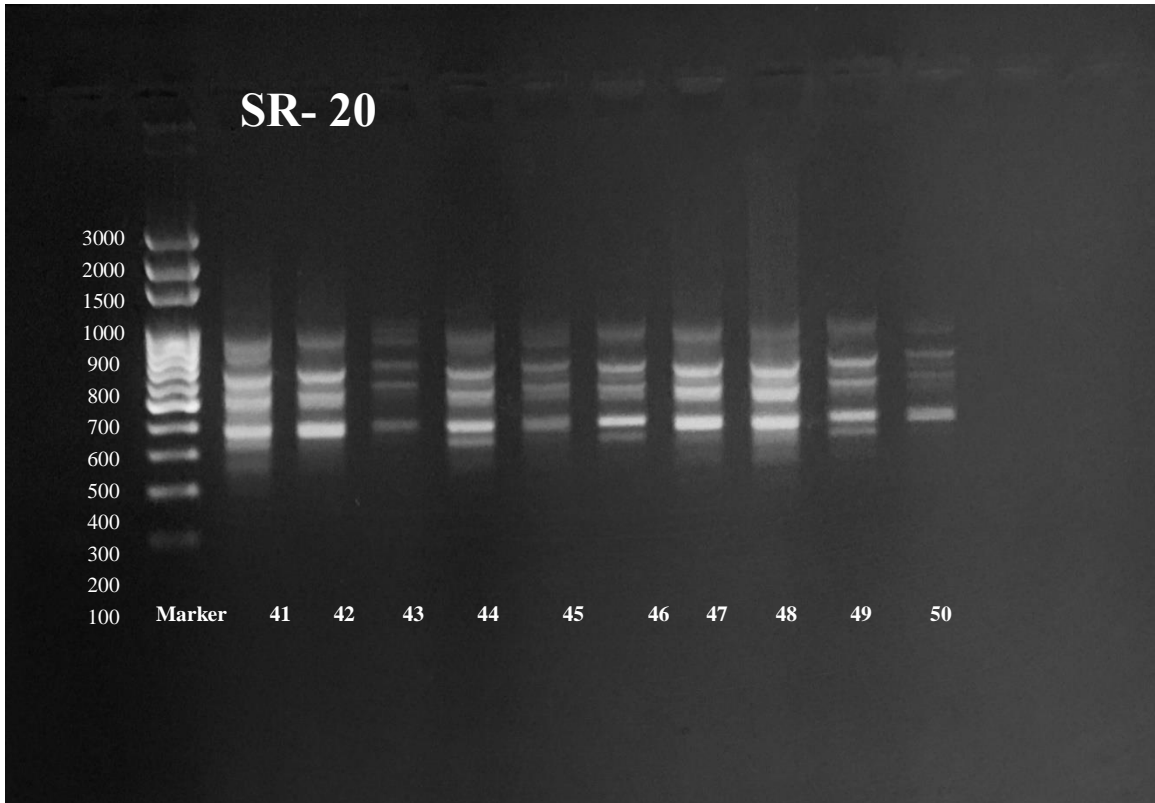


Figure 4.28 Banding patterns of ISSR primer 20 for 10 sesame cultivars

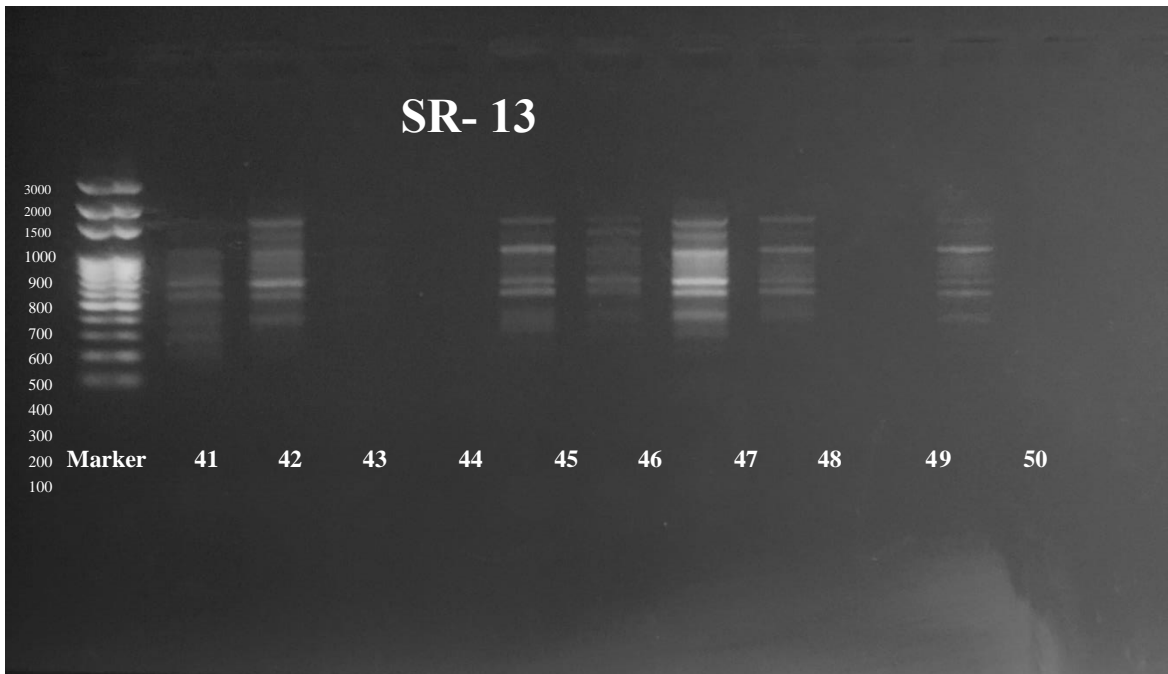


Figure 4.29 Banding patterns of ISSR primer 13 for 10 sesame cultivars

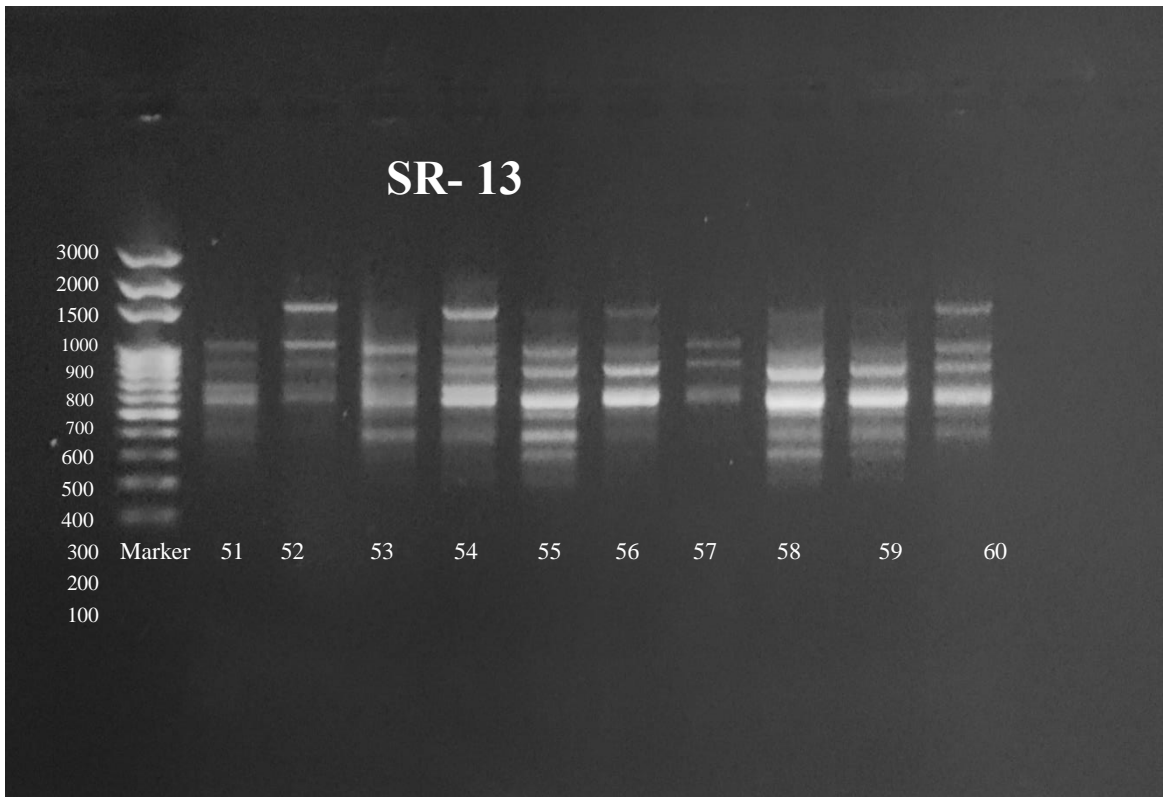


Figure 4.30 Banding patterns of ISSR primer13 for 10 sesame cultivars

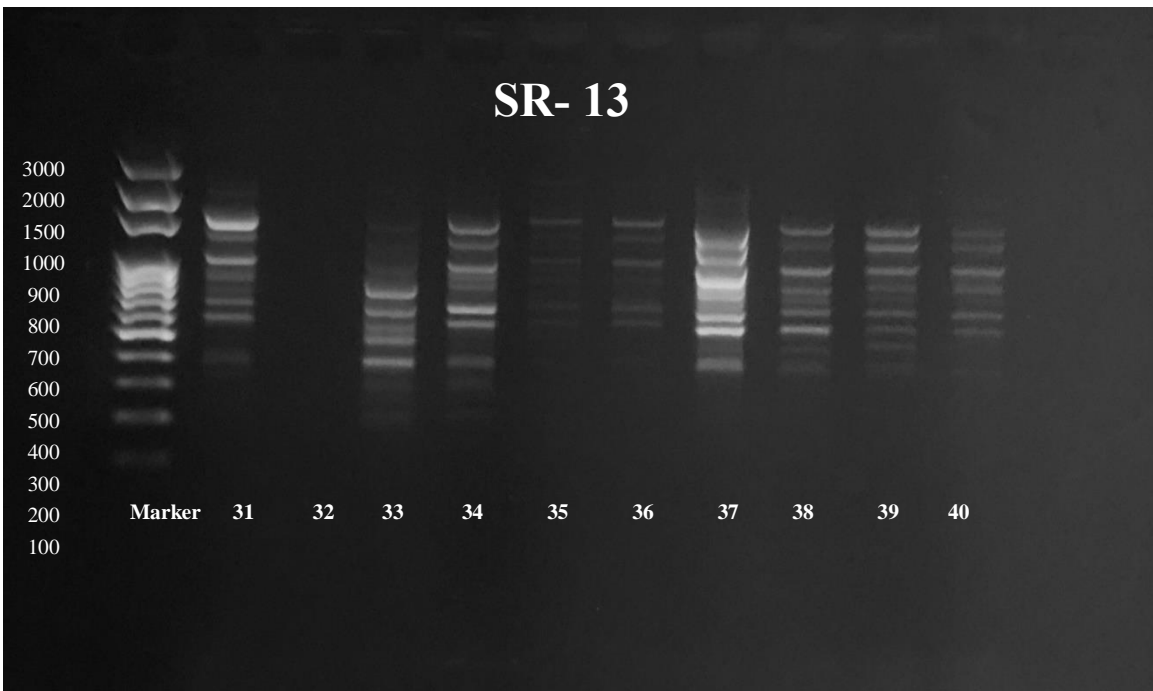


Figure 4.31 Banding patterns of ISSR primer 13 for 10 sesame cultivars

Chapter five

Conclusions & Recommendations

Chapter five

5.1 Conclusions

The study showed significant differences ($p \leq 0.05$) in the plant height (cm), number of branches per plant and number of capsules per plant. These variations were attributed to the origin of geographical as they were collected from different areas of Sudan tis. South and north kordfan, darfour, Blue Nile and gadarif. The significant differences between the cultivars in these parameters were observed by many researchers. El naim *et al* (2012) reported significant differences in plant height among different sesame genotypes. Also the excrement showed that the plant ranged between (1.0 – 2.0) meters which agree with Bennett et al 2003 were reported the same range dependence on the cultivar and the growing conditions. Abdalla, 2003 and Nagla *et al* 2013 reported difference in number of branches per plant.

The number of branches per plant were recorded to be in the range of 2 to 8 branches/ plant. Many researchers reported closer range of the number of branches per plant, (4 – 8), Geremew *et al* (2012) who reported that the number of branches per plant is affected by seed rate, rainfall, the day length and the variety of sesame. This study attributed this variation to the origin of the seed source.

Although this study and many researcher's studies showed the significant difference in the number of capsules per plant. Abdallah, (2017) observed non-significant difference between sesame genotypes.

As stated by (sogut, 2008), these variation of number of capsules per plant among genotypes may be differenced to the adaptation to the day length which

affect the number of branches per plant which can be seriously affect by the climatic conditions.

Seed germination percentage was significantly ($p \leq 0.05$) decreased by increasing the abiotic stress. increasing sodium chloride from zero to 60 and 80 mM causing salinity and poly ethylene glycol from zero to 6 and 9 bar causing drought stress decreased germination percentage in all the sesame. Tested with the same variations between varieties which attributed to the origin of the seeds under test.

Seedling shoot and root length were affected by salinity and drought. The significantly ($p \leq 0.05$) decreased as result of increasing either salinity or drought stresses. Increasing Nacl from zero to 60 and 80 mM and PEG from zero to 6 and 9 bar showed the significant decreased in the shoot and root length. The varieties different in their response to either salinity stress or drought stress. These difference were attributed to the origin of the seeds.

As a result of the standard germination test using the germinator curve the germination speed and germination parameters were illustrated by histogram and boxplot which showed that the total germination percentage ranged between zero and 100% in the control untreated seeds. Within this range 80% showed normal germination seeds.

Drought stress decreased the seeds germination percentage in the range of zero to 60% germination at dt1 with no differences between dt1 and dt2. Whereas the germination percentage decreased in the range of zero to 23% with significant differences between sl1 and sl2 mM to about 50 % less for sl2. Increasing salinity concentration resulted in more than 90% abnormal seed germination.

Almost all the tested seeds showed 100% germination with a maximum seed shoot length. Drought stress decreased the shoot length as only 50% of the seed reached 80% of the maximum shoot growth at dt1 with a decreased to 40% of the seed reaching 60% of shoot growth at dt2.

Salinity stress decreased the root length with increasing NaCl concentration from sl1 to sl2 in the 90% growth with 80% of the seed with sl1 to 70% growth at 60-80% of the seeds shoot length.

All the tested 60 cultivars reached the the maximum shoot length frequency at almost 100% . 70% frequency of the cultivars have a root length 80% percentage at the drought concentration (dt1) compare to 50% shoot length. About 60% of the cultivars have about (40-60)% root growth at (dt2).

53% of cultivars reached a maximum root length at 60% germination with increasing the concentration to (sl2) resulted in decreasing the frequency of root length at 80 and 100% with the maximum of more than 50% of the cultivars showing 40% of root length.

Germination had been recorded daily for 5 days and data were used to analyse germination speed i.e. the time required to reach 10% germination (T10) , the time required to 50 % germination (T50) and the area under the germination time curve after 150 hours (AUC).

Almost all the tested seed seed reached 10% germination in a time of about 135 hours (40%) from the beginning of the experiment. few variation were observed among the treatments compared to the control.

As in the T10 and T50 reached the maximum germination in (135-175) hours (40-60)% depending on the treatment. The variation in the time taken for the seed germination was attributed to sesame genotypes tested as observed in

the field experiment. The area under the curve (AUC) increased with increasing time regardless of the treatments.

Many research workers studied the effect of abiotic stresses especially drought and salinity on sesame germination and development. Ma, et al. 2020 reported that the response of plants to abiotic stress depends on many factors such as the species and genotypes. Kumar, et al. 2001 studied the effect of drought on crop growth. Singh, et al. 2001 reported the effect of salinity stress on plant growth.

These studies conducted that increasing salinity or drought stress significantly decreased plant growth i.e. seed germination, seedling shoot and root length.

In ISSR analysis, the number of amplified products (bands) obtained was specific to each primer (Table 4.2) and ranged from 60 accessions. The number of polymorphic bands per primer in the present study was 100%. The evaluated 10 ISSR primers produced a total of 107 bands, of which 107 bands were polymorphic. The molecular weight of the amplified fragments ranged from 200 bp to 3000 bp. The PIC values for the evaluated primers were relatively high (0.362593), (Table 4.2) and validity of the selected primers. Primer 23 showed the highest PIC value (0.362593), whereas primer 20 had the lowest PIC value (0.214444). These results disagree with (Singh, Kumar, and Kumar 2015). They said that in ISSR analyses, a total of 183 alleles were detected among which 164 alleles (89.61%) were polymorphic. The number of alleles per primer ranged from 2 to 15 with an average of 7.32 alleles per ISSR primer. In addition, 12 selected SSR primers generated 41 amplified bands. The fragment size varied from 155 to 371 bp.

UPGMA analyses were used to construct a dendrogram for the 60 genotypes of sesame cultivars based on the 107 bands obtained from 10 primers. were divided into two major groups (I, II) Depend on geographical distribution (west and east of Sudan) respectively, group (I) was sub-grouped(A&B) into 4 clusters depend on the area west Sudan which divided in to Darfur and kordfan.

The poor correlation between stresses and molecular marker in all accession of sesame based analysis was indicated by explain Regression Test R Square less than 0.5.

5.2 Recommendation

1. Molecular characterization of sesame genome (Genotyping) using one of the most advanced molecular markers SNP (Single Nucleotide Polymorphism markers).
2. Detect the major loci, marker trait associations (MTA), or putative genes that associated with seed longevity in sesame using the new technology Genome Wide Association Studies (GWAS).

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