Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576 e-ISSN: 1308-7584

Research Article

# Evaluation of Sesame (*Sesamum indicum* L.) Lines Under Salt Stress for Seed Yield Using SSR Markers

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**Article Info**

Received: 17.08.2022
Accepted: 13.06.2023
Online published: 15.09.2023
DOI: 10.29133/yyutbd.1163062

**Keywords**

Salinity,
Seed yield,
Sesame,
SSR markers

**Abstract:** Salinity has undesirable effects on sesame yield. In order to reduce salt's harmful effects, sesame tolerance needs to be increased. Twenty-three lines of sesame were irrigated with saline water (70 and 90 mM NaCl) and evaluated based on seed yield over two seasons (2019–2020). Genotypes were evaluated in a randomized complete block design (RCBD) with three replications. Ten SSR molecular markers were used to evaluate these lines for salt tolerance. Genotypes showed significant differences ($p < 0.05$) and recorded a wide range of seed yields under optimum and salinity conditions. Four lines (C1.5, C2.2, C8.4, and C9.15) achieved the highest average performance for seed yield compared to other lines under salinity conditions. Ten SSR markers revealed 15 alleles, ranging from 1 to 4 alleles. The polymorphism information content (PIC) ranged from 0.00 to 0.44. The range of expected heterozygosity (He) was 0.00 to 0.444. The UPGMA dendrogram analysis divided all sesame genotypes into two main clusters. In addition, SSR 3 and SSR 6 markers elucidated the possibility of using them in breeding programs for enhancing salt tolerances in sesame cultivars. These lines may be used as a salt-tolerant source in future breeding to create new sesame cultivars.

**To Cite:** Samaha, G M, Sayed, L M, Saber, A A, 2023. Evaluation of Sesame (*Sesamum indicum L.*) Lines Under Salt Stress for Seed Yield Using SSR Markers. *Yuzuncu Yil University Journal of Agricultural Sciences*, 33(3): 397-407. DOI: https://doi.org/10.29133/yyutbd.1163062

## 1. Introduction

The sesame (*Sesamum indicum* L.) crop has many advantages, including stress tolerance, its composition of amino acids matches animal protein, thus its seed protein is outstanding to other oil crops (Boureima et al., 2011). Sesame seeds are contained protein, vitamin B1, manganese, phosphorous, copper, calcium, manganese, iron and zinc, and fiber, so their seed is a high nutritional value. The oil contains sesaminol and sesamin lignans that play an important role in the activity of tocopherols and other antioxidants (Lee et al., 2008).

Sesame is more adaptable to a broad range of soil types (Islam et al., 2016). This quality raised sesame as an attractive crop specially designed for challenging climatic changes (Li et al., 2018). Although

salt stress is a serious factor affecting productivity (Bahrami et al., 2016; Zhang et al., 2020). Meanwhile, the shortage of freshwater resources in Egypt poses a major threat to agricultural production in the present and the near future. Moreover, Egypt imports most of its vegetable oils. In general, cultivated sesame in Egypt encounters several stress factors including salinity and drought, which limited its productivity. So, growing sesame cultivars that can withstand salinity is a very significant option to fix this problem and reduce oil imports. Moreover, increasing the area planted for sesame crops to contribute to covering the need for edible oil (El-Hamidi and Zaher, 2018). Also, the development of plants that can withstand salt supports sustainable agriculture and offers a longer strategy to manage salt-affected soils and less impact on seed quality and yield becoming a hard mission for breeders (Qin et al., 2020).

To assess sesame genetic variability and identify new genetic sources for biotic and abiotic stress tolerance, phenotypic and molecular analyzes are combined (Bekele et al., 2017; Bose et al., 2017). There are genetic factors that control the diversity in sesame responses to salt stress. Consequently**,** the detection of QTLs and candidate genes associated with these characters will be important to speed the development of abiotic tolerance breeding in sesame. (Dossa et al., 2017). Therefore, many various molecular markers were used to estimate the genetic diversity of sesame and to detect associated genetic markers with salt-tolerance traits (Dossa et al., 2016; Wei et al., 2016; Asekova et al., 2018; de Sousa Araújo et al., 2019; Stavridou et al., 2021).

Among molecular markers, SSR markers are characterized by multi-allele nature, co-dominant inheritance, distribution in the genome, and reproducibility (Baruah et al., 2019). In comparison to other genetic markers, SSR markers provide more information about genetic diversity (Wei et al., 2014; Baruah et al., 2019). Dossa et al*.* (2016) identified 91 SSR markers related to the AP2/ERF genes in sesame. These SSR markers are useful for marker-assisted selection (MAS) to improve sesame toward abiotic stresses.

Our study aimed to evaluate new lines of sesame based on seed yield under salt stress and using SSR markers to confirm the salinity tolerance of these lines.

## 2. Material and Methods

### 2.1. Description of the study area

Genotypes were evaluated based on seed yield $ha^{-1}$ under two concentrations of sodium chloride (70 and 90 mM) in an open field in sandy conditions, and we used two tanks (1 $m^3$) for irrigation and we used drip irrigation. Irrigation was weekly. The experiments were applied for two years (2019 and 2020) at the Research and Production Station, National Research Centre, Al-Nubarya, El-Behira Governorate (latitude 30° 30\ N, and longitude 30° 19\ E, and mean altitude 21 m above sea level).

### 2.2. Breeding materials

The twenty-three new lines of sesame were obtained from the Department of Agronomy, Faculty of Agriculture, Cairo University, Egypt (Table 1.). C1.3, C1.5, C1.6, C1.8, C1.9, C1.10, C2.2, C2.3, C2.6, C3.4, C3.8, C5.7, C6.3, C6.5, C6.7, C6.9, C8.4, C8.8, C8.11, C9.6, C9.7, C9.15, and C9.20 are the names of the lines. And two check cultivars (cv. Shandweel and Sohag) were obtained from the Ministry of Agriculture and Land Reclamation, Egypt.

### 2.3. Design of an experiment

Randomized complete block design (RCBD) with 3 replicates was utilized for each concentration. Plots are composed of 1 row that is 1.5 m long, spaced 70 cm apart, and planted at a distance of 10 cm. Reservoirs were supplemented with NaCl (10 $m^3$) to irrigate the field's rows. The recommendation of the Ministry of Agriculture was used. Seed yield $ha^1$ of the samples from the three replications' net areas (1.04 $m^2$) was collected. Recommended agricultural practices were used to cultivate the genotypes. According to Saber (2015), Table 1 displayed the parents' descriptions. Variance analysis was calculated by the computer program MSTAT-C (MSTAT-C program, 1991).

Table 1. Source of new sesame lines according to their breeding status and parents' traits

| Parents | Breeding status | Source of seeds * | Specific traits |
|---|---|---|---|
| **P1 (HM19)** | $F_8$-hybrid pop | Cairo Univ.* | Early maturity, three capsules per axil, first capsule set low, non-branching, resistance to *Fusarium Oxysporum is very high.* |
| **P2 (EUL90)** | Mutant line | Cairo Univ.* | Non-branching, premature, the base of first capsule low, three capsules per axil, moderately resistant to *Fusarium Oxysporum.* |
| **P3 (Mutant48)** | Mutant line | Cairo Univ.* | Branching, high susceptibility to *Fusarium oxysporum,* number capsules per axil three |
| **P4 (Giza 32)** | Local variety | Ministry of Agric.& Land Reclamation, Egypt | Heavy seed weight, medium branching, one capsule/axil, long capsule, late maturity, resistance of *Fusarium Oxysporum* is moderate |
| **P5 (NM59)** | Exotic line | India through IAEA** | *Fusarium oxysporum*-resistant, rigid stem, late maturity, one capsule/axil |
| **P6 (Babil)** | Exotic variety | Iraq through IAEA** | Decreased branching, semi- shattering capsules, 3 capsules/axil, resistant to *Fusarium Oxysporum* |

*Advanced breeding materials resulted from the breeding program conducted at Agron. Dept. Fac. Of Agric. Cairo Univ.
**Inter. Atomic Energy Agency.

## 2.4. Genotypic analysis

Nine lines from 23 were selected for SSR analysis according to mean performance for seed yield $ha^{-1}$ under salt stress. The following nine lines: C1.5, C1.6, C2.2, C3.8, C6.3, C6.5, C8.4, C8.8, and C9.15 to be compared with two check cultivars (Shandweel and Sohag) of sesame. Utilizing the DNeasy Plant Mini Kit and the manufacturer's recommendations, DNA was extracted from young fresh leaves (Qiagen). Genomic DNA was loaded in 0.8% agarose gel and separated by electrophoresis for 60 min at 100 volts.

### *2.4.1. SSR-PCR analysis*

Ten SSR primers (Table 2.) were used for the amplification among eleven sesame genotypes to be utilized as markers for screening sesame lines differing in salinity response. In this study were identified four SSR primers (from SSR 1 to SSR 4) based on the salt-responsive candidate gene (cg-SSR) which was published by Li et al. (2018). Using an online SisatBase database, (http://www.sesame-bioinfo.org/SisatBase/) and (http://www.sesame-bioinfo.org/PMDBase) following BLAST (https://blast.ncbi.nlm.nih.gov/). While the other 6 primers (from SSR 5 to SSR 10) were selected from 91 SSR markers from a published source (Dossa et al., 2016).

Each 10µL of PCR mixture for the amplification of SSR bands consisted of 5 µL (2X) of KAPA2G Fast Ready Mix[2] (KK5101), a 0.5µL of forward primer, a 0.5µL of reverse primer, a 1µL of DNA template and H2O up to 10 µL. Amplification was performed on a Primus thermal cycler, programmed for 37 cycles as follows; Initial denaturation, 95°C/4 min (one cycle), denaturation 94°C/1 min, annealing, 58°C /45 sec, extension 72°C/ 1.5 min (35 cycles), final extension, 72°C/10 min (one cycle), then kept at 4°C until use. The amplification product was separated on agarose (3%) by electrophoresis. The UV-transilluminator filter was used to see the DNA bands in the gel. A digital imaging device was used to take pictures of the bands. Solis BioDyne 100 bp DNA Ladder (07-11-00050) was employed as a size marker. M (100 bp Ladder DNA), 1 ( C1= Shandweel), 2 (C2 = Sohag), 3 (C1.5), 4 (C1.6), 5 (C2.2), 6 (C3.8), 7 (C6.3), 8 (C6.5), 9 (C8.4), 10 (C8.8) and 11 (C9.15) respectively.

### *2.4.2. Analysis of gel images*

Gel images were analyzed using Total lab TL 120 to determine the molecular size of amplified fragments. Amplified fragments were classified as present (1) or absent (0). Polymorphic Information Content (PIC), Expected Heterozygosity (He), and Effective Multiplex Ratio (EMR) values were determined using the online program (https://irscope.shinyapps.io/iMEC/) according to Amiryousefi et al. (2018). The NTSYS program was used to construct the dendrogram (Rohlf, 2000).

Table 2. SSR primers, gene name, candidate gene ID, forward sequence, and reverse sequence

| SSR no. | Gene Name | Candidate Gene ID | Forward sequence | Reverse sequence |
|---|---|---|---|---|
| **SSR 1** | SiGPAT3 | SIN_1007701 | ACAAAGCTCACGAGGAAGGA | CATGCACTTTTACCGCAGTG |
| **SSR 2** | SiMLP31 | SIN_1021337 | CCAACTCGTCCGCACATAAT | ATGCCACCCAAGAAATTGAG |
| **SSR 3** | SiGRV2 | SIN_1001572 | CGTCGAATCATATTGGAGCA | GTGAACTTGAAGGCCTCTGC |
| **SSR 4** | SiGRF5 | SIN_1024695 | TACAGGCACACCAGAAACCA | ATGAGTGGTGGTGGGAGAAG |
| **SSR 5** | AP2si2 | SIN_1009557 | CCGTCGTGCTCGTCTTCT | CGGATTCAGCCACCCTTC |
| **SSR 6** | AP2si11 | SIN_1013899 | CTCCTCATCGGACTCTTC | GCGTCTTCATTCCCACT |
| **SSR 7** | AP2si16 | SIN_1017978 | TCTTGCGAATTAGAAGGC | ACTCACATTTATTACCACCATC |
| **SSR 8** | AP2si90 | SIN_1010530 | TCCATCGTCCTCCCATCA | AAACATCGCCTCCTCGTC |
| **SSR 9** | AP2si106 | SIN_1008520 | CTCCACCTCTTCGCCGTCTG | CGCCCTTATCATCTTCTTCTGC |
| **SSR 10** | AP2si116 | SIN_1003959 | CACAGCCGTGTACTACCTCC | TGCCGCCTTCTTCCTTAT |

## 3. Results and Discussion

### 3.1. Mean performance and variance

Table 3 shows the results of a statistical analysis of the sesame genotypes for seed yield under various conditions. Sesame genotypes varied significantly ($p > 0.05$) in terms of seed productivity. And they showed a wide range of seed yield under different conditions, suggesting that some of these genotypes may be tolerant to salt conditions, which reflected positively on selection for salinity tolerance. Similar results were reported by Bahrami et al. (2016), Anter and El-Sayed (2020), and Dangue et al. (2022). The variable performance of genotypes was due to their genetic make-up, which caused the lines to respond differently when exposed to salt levels, similar results were noted by Suassuna et al. (2017).

In this study, line C5.7 achieved the highest seed yield (1171.8 kg $ha^{-1}$) followed by C1 (1079.7 kg $ha^{-1}$) and C2.6 (1004.4 kg $ha^{-1}$) under normal conditions.

On the other hand, we found that the four lines C1.5, C2.2, C8.4, and C9.15 performed better than the control cultivars (C1 and C2) and other lines in terms of seed yield in salt conditions. These lines may sense the expression of salt-stress-responsive genes, which regulate processes including detoxification, ion transport, and osmotic balance. Numerous regulatory elements, including phytohormones, lipids, the cell wall, and the cytoskeleton, are used by these mechanisms (Van et al., 2020; Gong, 2021).

According to CR% values (rate decrease in seed yield under salinity conditions compared to seed yield under normal conditions), fives lines C8.4, C8.8 C3.8, C6.3, and C8.11 were less affected by salinity conditions compared to check cultivars despite of were less seed productive under normal condition.

In general, the seed yield of all genotypes was affected by salinity conditions. The adverse effects of poor irrigation water quality on genotypes are evident may be due to the inhibition some of biochemical, and physiological processes, and ion imbalance (Dias et al., 2017; Shahid et al., 2020). In addition, the line's ability to absorb nitrogen is reduced under salinity conditions (Saha et al., 2015).

Table 3. Seeds yield $ha^{-1}$ of sesame genotypes under normal and salinity conditions

| Genotypes | 70mM NaCl | 90mM NaCl | Mean seed yield under salinity conditions ($\bar{X}$) | Seed yield under normal condition | Change rate in seed yield under salinity conditions (CR %) |
|---|---|---|---|---|---|
| **C1.3** | 160.5±2.21 | 130.5±1.83 | 145.5 | 948.6±12.6 | 84.7 |
| **C1.5** | 187.0±2.65 | 152.0±2.13 | 169.5 | 788.6±10.5 | 78.5 |
| **C1.6** | 177.5±2.49 | 144.4±2.03 | 160.9 | 669.6±8.9 | 76.0 |
| **C1.8** | 151.1±1.14 | 123.1±0.93 | 137.1 | 558.5±7.4 | 75.5 |
| **C1.9** | 134.4±1.89 | 109.7±1.54 | 122.0 | 651.0±8.6 | 81.3 |
| **C1.10** | 158.9±1.2 | 129.5±0.98 | 144.2 | 684.4±9.1 | 78.9 |
| **C2.2** | 192.2±2.7 | 156.2±2.19 | 174.2 | 892.8±11.8 | 80.5 |
| **C2.3** | 167.0±2.34 | 136.0±1.91 | 151.5 | 688.2±9.1 | 78.0 |
| **C2.6** | 161.6±2.77 | 131.2±2.25 | 146.4 | 1004.4±13.3 | 85.4 |
| **C3.4** | 122.9±0.93 | 99.9±0.75 | 111.4 | 703.0±9.3 | 84.2 |
| **C3.8** | 171.2±2.4 | 138.8±1.95 | 155.0 | 587.7±7.8 | 73.6 |
| **C5.7** | 113.1±0.85 | 92.2±0.7 | 102.6 | 1171.8±15.5 | 91.2 |
| **C6.3** | 176.4±2.47 | 143.7±2.02 | 160.0 | 613.8±8.1 | 73.9 |
| **C6.5** | 171.6±1.3 | 139.2±1.05 | 155.4 | 892.8±11.8 | 82.6 |
| **C6.6** | 150.2±1.13 | 122.5±0.93 | 136.3 | 613.8±8.1 | 77.8 |
| **C6.7** | 127.7±0.96 | 103.8±0.78 | 115.7 | 628.6±8.3 | 81.6 |

Table 3. Seeds yield ha$^{-1}$ of sesame genotypes under normal and salinity conditions (continued)

| Genotypes | 70mM NaCl | 90mM NaCl | Mean seed yield under salinity conditions ($\bar{X}$) | Seed yield under normal condition | Change rate in seed yield under salinity conditions (CR %) |
|---|---|---|---|---|---|
| **C8.4** | 191.8±3.29 | 156.2±2.68 | 174.0 | 591.4±7.8 | 70.6 |
| **C8.8** | 178.6±3.06 | 144.9±2.48 | 161.8 | 610.0±8.1 | 73.5 |
| **C8.11** | 170.1±2.92 | 138.7±2.38 | 154.4 | 598.9±7.9 | 74.2 |
| **C9.6** | 160.7±2.75 | 130.6±2.24 | 145.6 | 747.7±9.9 | 80.5 |
| **C9.7** | 149.3±2.56 | 121.2±2.08 | 135.2 | 788.6±10.5 | 82.9 |
| **C9.15** | 182.3±1.38 | 148.2±1.12 | 165.8 | 967.2±12.8 | 82.9 |
| **C9.20** | 120.0±2.06 | 97.4±1.67 | 108.7 | 788.6±10.5 | 86.2 |
| **C1** | 120.5±3.0 | 124.5±4.5 | 122.7 | 1079.7±14.1 | 88.6 |
| **C2** | 100.0±2.7 | 104.1±1.17 | 102.1 | 900.0±12.0 | 88.7 |
| Significant level **(P<0.05)** | 88.0 | 63.1 | - | 490.0 | - |
| **Coefficient of variation (CV%)** | 5.3 | 3.0 | - | 14.8 | - |

±: Stander error, C1: Shandweel, C2: Sohag.

### 3.2. Rank genotypes

To make a good judgment on the extent to which the current study materials are affected by environmental conditions, we ranked genotypes based on mean performance for seed yield, ranks mean, and stander deviation under different conditions (Table 4.). Lines with low overall rankings ($\bar{X}$) were regarded as generally adaptive to salinity conditions and distinguished from others. Abate (2015) pointed out that.

Table 4. The rank of sesame genotypes under normal and salt conditions

| Genotypes | 70mM NaCl | 90mM NaCl | Seed yield ha$^{-1}$ (kg) under normal condition | Ranks mean ($\bar{X}$) | Standard deviations (Sd) |
|---|---|---|---|---|---|
| **C1.3** | 14.0 | 14.0 | 5.0 | 11.0 | 5.2 |
| **C1.5** | 3.0 | 4.0 | 8.0 | 5.0 | 2.6 |
| **C1.6** | 6.0 | 6.0 | 15.0 | 9.0 | 5.2 |
| **C1.8** | 5.0 | 17.0 | 25.0 | 15.7 | 10.1 |
| **C1.9** | 19.0 | 20.0 | 17.0 | 18.7 | 1.5 |
| **C1.10** | 15.0 | 16.0 | 15.0 | 15.3 | 0.6 |
| **C2.2** | 1.0 | 1.0 | 7.0 | 3.0 | 3.5 |
| **C2.3** | 11.0 | 11.0 | 14.0 | 12.0 | 1.7 |
| **C2.6** | 12.0 | 12.0 | 3.0 | 9.0 | 5.2 |
| **C3.4** | 21.0 | 23.0 | 13.0 | 19.0 | 5.3 |
| **C3.8** | 9.0 | 9.0 | 25.0 | 14.3 | 9.2 |
| **C5.7** | 24.0 | 25.0 | 1.0 | 16.7 | 13.6 |
| **C6.3** | 7.0 | 7.0 | 19.0 | 11.0 | 6.9 |
| **C6.5** | 8.0 | 8.0 | 9.0 | 8.3 | 1.0 |
| **C6.6** | 17.0 | 18.0 | 20.0 | 18.3 | 1.5 |
| **C6.7** | 20.0 | 22.0 | 18.0 | 20.0 | 2.0 |
| **C8.4** | 2.0 | 2.0 | 23.0 | 9.0 | 12.1 |
| **C8.8** | 5.0 | 5.0 | 21.0 | 10.3 | 9.2 |
| **C8.11** | 10.0 | 10.0 | 22.0 | 14.0 | 6.9 |
| **C9.6** | 13.0 | 13.0 | 12.0 | 12.7 | 0.6 |
| **C9.7** | 18.0 | 19.0 | 10.0 | 15.7 | 4.9 |
| **C9.15** | 4.0 | 3.0 | 4.0 | 3.7 | 0.6 |
| **C9.20** | 23.0 | 24.0 | 11.0 | 19.3 | 7.2 |
| **C1** | 22.0 | 16.0 | 2.0 | 13.3 | 10.3 |
| **C2** | 25.0 | 21.0 | 6.0 | 17.3 | 10.0 |

C1: control 1 (Shandweel cultivar), C2: control 2 (Sohag, cultivar).

Line C2.2 achieved a low-rank mean (3.0), and a low value of standard deviation (3.5), and was ranked first under salinity conditions while being categorized as seventh under normal conditions. As shown by rank mean and low standard deviation, it also showed little variation in relative performance across environments. Line C9.15 was ranked fourth at 70 mM, and third at 90 mM. It has been categorized as fourth under normal conditions. It also achieved a low-rank mean (3.7) and a low value of standard

deviation (0.6). Line C1.5 is ranked third at 70 mM and fourth at 90 mM and it achieved a low-rank mean of 5.0 and a middle value of the standard deviation of 2.6 and it was ranked eighth under normal conditions.

The results highlighted the ability of these lines to adapt to salinity conditions. These lines may possess useful genetic factors to increase salt tolerance, as suggested by Zhang et al. (2019) in rice crops. Additionally, these lines may be able to resist water stress and/or be tolerant to ion toxicity as indicated by Shrivastava and Kumar (2015), and/or they may produce osmols such as organic acids, soluble sugars, free amino acids and increased accumulation of potassium ions (Parvaiz et al., 2012). Here appear the role of the crops breeder, through the combination between salt tolerance and a high seed yield potential as bio-ameliorators.

### 3.3. Genetic polymorphism of the SSR markers

Molecular markers are used in breeding programs to improve their efficiency and effectiveness. Simple sequence repeats (SSRs) are a considerably effective technique for identifying crop varieties (Raghunath, 2022). Numerous studies were performed on the tolerance of drought and salt stresses in sesame including those by Bazrafshan and Ehsanzadeh, (2014), (2016) and Dossa et al. (2016). In addition, several studies indicate the important role of the AP2/ERF family of transcription factors (TFs) in plant biotic/abiotic stress tolerance (Akhtar et al., 2012; Mizoi et al., 2012; Chen et al., 2022). Furthermore, Dossa et al. (2016) determined 91 SSR markers related to the AP2/ERF genes in sesame. Li et al. (2018) found 27 candidate genes for salt responses helpful for enhancing salt tolerance in sesame cultivars.

Therefore, we used 10 SSR markers, from SSR 1 to SSR 4 were identified based on the salt-responsive candidate gene (Li et al., 2018), these SSRs were distributed on four chromosomes (chr5, chr2, chr4, and chr11), respectively. This in harmony with those obtained by Sharma et al. (2021) used the wheat genome to generate 177 heat-responsive gene-based SSRs for heat tolerance. The other six markers used in this study were selected according to Dossa et al. (2016) for evaluating the genetic variability of new lines for salinity tolerance.

The results revealed amplified fragments ranging from 66 bp to 1250 bp through the 10 SSR markers among 9 lines and two cultivars of sesame (Table 5.). Out of 10 SSRs screened, only two SSRs were polymorphic (SSR 3 and SSR 6) primers (20% polymorphism) as shown in Figure 1. This indicates that the number of polymorphic SSR primers was very low in this study. This point is agreed with and supported by Pandey et al. (2015) reported that only eight primers from thirty-six East-SSR were used to identify the accessions. Likewise, Ramprasad et al. (2017) used 75 SSR primer pairs, only 20 were polymorphic (29.4% polymorphism). The level of polymorphism was higher in our study compared to an earlier report by Yepuri et al. (2013) they found only 12 % of 156 primers polymorphic in a set of 49 sesame accessions consisting of germplasm.

A total of 15 alleles among the 11 sesame sample were observed (Table 5.). Each marker produced 1 to 4 alleles, with an average of 1.5 alleles per locus. The highest alleles number per locus was observed for SSR 3 (4 alleles) followed by SSR 6 (3 alleles). These results were lower than those reported by Pandey et al. (2015) reported that the number of alleles ranged from 2 to 6 alleles, with an average of 3.37 alleles per locus.

The PIC value varied from 0.00 to 0.35 with an average of 0.039. Similar results were also reported by Teklu et al. (2021) who found that the highest value of PIC in sesame was 0.37. The average of PIC (0.039) is lower than the values of 0.25 and 0.82 revealed by Teklu et al. (2021) and Stavridou et al. (2021) used 27 SSR markers and 28 EST-SSR markers to assess 100 and 35 sesame genotypes, respectively.

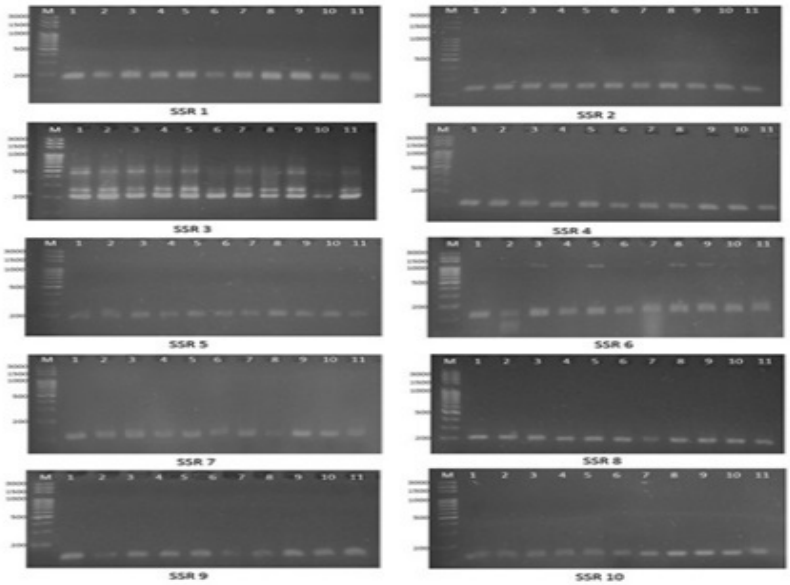


Figure 1. Amplification profile of SSR markers for eleven sesame genotypes.

To describe genetic diversity, expected heterozygosity (He) is usually used (Chesnokov and Artemyeva, 2015). In our study, the (He) ranged from 0 to 0.444 with an average of 0.049. This value was higher than reported by Ramprasad et al. (2017) who found the (He) ranged from 0.00 to 0.2162, with a mean of 0.0465 in 41 sesame genotypes. The mean expected heterozygosity (0.049) was lower than the value of 0.30, 0.34, and 0.72 reported by Teklu et al. (2021), Asekova et al. (2018), de Sousa Araújo et al. (2019) when evaluating 100, 129 and 36 sesame accessions using 27, 23 and 10 SSR markers, respectively.

Effective multiplex ratio (EMR) equals the total number of polymorphic loci for each primer multiplied by the rate of polymorphic loci from the total number (Nagaraju et al., 2001). The effective multiplex ratio varied from 1(SSRs 1, 2, 4, 5, 7, 8, 9, and 10) to 3.9 (SSR 3) with an average of 1.39.

Table 5. The results obtained from amplification with SSR markers

| SSR no. | No. of alleles | He | PIC | EMR | Product size bp |
|---|---|---|---|---|---|
| **SSR 1** | 1 | 0 | 0 | 1 | 175 |
| **SSR 2** | 1 | 0 | 0 | 1 | 234 |
| **SSR 3** | 4 | 0.044 | 0.043 | 3.9 | 223-614 |
| **SSR 4** | 1 | 0 | 0 | 1 | 126 |
| **SSR 5** | 1 | 0 | 0 | 1 | 207 |
| **SSR 6** | 3 | 0.444 | 0.346 | 2.0 | 66-1250 |
| **SSR 7** | 1 | 0 | 0 | 1 | 111 |
| **SSR 8** | 1 | 0 | 0 | 1 | 196 |
| **SSR 9** | 1 | 0 | 0 | 1 | 116 |
| **SSR 10** | 1 | 0 | 0 | 1 | 147 |
| **Total** | 15 | 0.488 | 0.389 | 13.9 | |
| **Average** | 1.5 | 0.049 | 0.039 | 1.39 | |

He: expected heterozygosity, PIC: Polymorphic Information Content, EMR: Effective multiplex ratio.

In the above, the details of the ten SSR primers were mentioned. But when excluding the monomorphic primers, we found the average values of PIC, He, and EMR for only two polymorphic primers (SSR3 and SSR 6) were 0.195, 0.244, and 2.95 respectively. In addition, the average number of alleles was 3.5 alleles per locus. SSR 3 showed the highest number of total bands (4) and the effective multiplex ratio (3.9), whereas SSR 6 gives the highest value of expected heterozygosity (0.444) and PIC values (0.346). This indicates that SSR 6 is more informative because the higher values of expected heterozygosity (He = 0.444) evidence that there is more allelic variation (Gaballah et al., 2021). And the PIC values (0.346) between 0.25 and 0.5 imply moderate levels of polymorphism for SSR 6 (Botstein et

al., 1980). The obtained results imply that SSR6 followed by SSR3 was more efficient in evaluating the new line for salinity stress in sesame.

Generally, these results show low genetic variation among genotypes because of the use of less number of primers. Or due to the selection of the SSRs linked to salinity tolerance, not random SSRs. A similar finding was also reported by Mir et al. (2012) and Shafi et al. (2021) detected less diversity by trait-specific SSRs compared to random genomic SSR markers in wheat.

### *3.3.1. Cluster analysis of genotypes*

The dendrogram was created based on the binary data obtained from the SSR marker-based DNA profiles of the genotypes examined (Figure 2.). Among 10 SSR markers, SSRs 3 and 6 were able to distinguish the genotypes into two main clusters based on their salinity tolerance. The first cluster involved C8.8 only. The second cluster was split into two sub-clusters, the first of which had Shandweel, C1.6, and C6.3, whereas the other of which had two sub-sub-clusters. Sub-sub-clusters I included only C2. On the other hand, sub-sub-clusters II consisted of the last six lines C1.5, C2.2, C9.15, C3.8, C8.4, and C6.5.

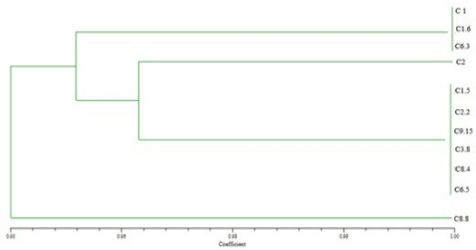


Figure 2. UPGMA dendrogram of the eleven genotypes based on the genetic similarity matrix.

## Conclusion

The study showed sesame lines differed significantly in terms of seed yield $ha^{-1}$ under different conditions, which indicated the possibility of obtaining genotypes that are tolerant to salinity conditions. Four lines C1.5, C2.2, C8.4, and C9.15 recorded the higher seed yield ha-1 under salinity conditions. Two lines C5.7 and C2.6 recorded the higher seed yield under normal conditions. SSR markers especially SSR 3 and SSR 6 were effective in screening salt tolerances in sesame cultivars. These markers would be useful in sesame breeding towards abiotic stresses. Finally, if we want to combine the results from field and genetic analysis there was an obvious similarity between the four lines C1.5 C2.2, C8.4, and C9.15 which reflect more tolerance to salinity and clustered in one group according to the UPGMA dendrogram.

## Acknowledgements

Thanks to the National Research Centre and the Science and Technology Development Fund for supporting this research (grant numbers 110 30127 and 34898).

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