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# GENETIC RELATIONSHIPS AMONG TEN SUNFLOWER INBRED LINES BASED ON ISSR AND RAPD ANALYSES

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## ABSTRACT

***The present investigation was conducted to access the genetic diversity among ten inbred lines of sunflower: Ha89, Ha93, Ha64, Ha101, Ha122, Sha13, Sha14, Sha15, Nsha136 and Nsha140 imported from different origins using RAPD and ISSR techniques. A total of 95 major scorable bands ranging from 117 to 1075 bp were generated from 8 ISSR primers showing 78.8% polymorphism. While, a total of 150 scorable bands ranging from 129 to 2835 bp were produced from 10 RAPD primers detecting 91.3 % polymorphism. Unique bands were produced by both ISSRs and RAPDs. Genetic similarity values were ranged between 51.1- 84.1 % and 38.9- 82.4 % for ISSR and RAPD, respectively. The lowest similarity using ISSR primers was observed between Sha14 and Ha93. While, the lowest similarity using RAPD primers was found between Ha89 and Nsha140. The dendrogram based on ISSR data separated the ten inbred lines into two main clusters at a similarity coefficient of 0.25, while, the dendrogram related to RAPD data separated them into three main clusters. The combined data of RAPD and ISSR showed genetic similarity ranged from 51.0 to 77.8 %. Also, the dendrogram based on the combined data of both ISSR and RAPD displayed considerably similar results to those obtained from individual ISSR analysis.***

Key words: ***Sunflower, Helianthus annuus* L. *Inbred line, Molecular markers, Genetic diversity, ISSR and RAPD.***

## INTRODUCTION

Sunflower is a global oilseed crop of economic importance. It is the second largest hybrid crop after maize (Hu *et al* 2010 and Seiler *et al* 2017) and the fifth oil seed crop after soybean, rapeseed, cotton seed and groundnut, where grown on 22.9 million hectares in 60 countries worldwide that considered it as the main source of edible oil such as America, Russia, Argentina, China, France, Spain, Romania, South Africa, Turkey, Serbia and Canada (Hu *et al* 2010). Sunflower oil has good effects on human health that lowers cholesterol levels in the blood. It contains antioxidants that prevent cancer. Also, it is an important source of the polyunsaturated fatty acid and protein. Mean of oil percentage of its seeds ranged from 35 to 50% and is rich in the desired fatty acide oleic (ca: 20-25%) as reported by (Premnath *et al* 2016). Aside from the use of sunflower and its oil in human nutrition, it is also used in different industries such as; biofuel, lubricants, surfactants and polymer synthesis (Dimitrijević *et al* 2017).

Sunflower occupies the fourth place in the production of edible oil globally, where its production reaches to 12 % (Rauf *et al* 2017). The total world production of major oil seeds in years 2016, 2017 and 2018 reached 572.84, 575.35 and 600.47 million metric tons, respectively. On the other

hand, Egypt imported 2.18, 3.33 and 3.42 million metric tons of oil seeds in years 2016, 2017 and 2018, respectively (USDA 2018). The major problem facing oil production in Egypt is the wide gap between production and consumption that production covers less than 10% of national consumption. In 2016 year, Egypt, Lebanon, Syria, Morocco, Tunisia, Sudan, Iraq and Palestine were the main producer for sunflower yield among Arab countries. Although sunflower the main oil crop cultivated in Egypt, Egypt imported 166.30 thousand tons of sunflower oil in price of 272.67 million dollars in a year 2016 (AOAD 2017). Many efforts have been made to solve this problem and recent discoveries have also led to the creation of appropriate solutions.

Sunflower belongs to the *Helianthus* genus (*Asteraceae*) is diploid species (2n= 34). Lately after using the genetic markers, it becomes easy to identify the superior parents and good combinations for heterosis breeding. Genetic diversity dramas an important role in plant breeding because hybrids between lines of diverse origin generally display a greater heterosis than those between closely related parents and this has become easy to predict before using breeding methods through cluster analysis based on molecular markers like RFLP, AFLP, RAPD, IRAP, REMAP, SSR and ISSR. Molecular genetic markers are extensively used in such cultures including sunflowe breeding that availability in time and effort. New methods of DNA fingerprinting can contribute to overcome the problems of narrow genetic diversity and will result more effective sunflower breeding program. Furthermore, as a result of using of genetic markers in various genetics branches, it has become a vital part of genetics particularly in genetic diversity and variability research (Kalendar *et al* 2017).

Relying on the importance of molecular markers, the present study was conducted to assess the genetic diversity among ten elite parents of sunflower using ISSR and RAPD markers.

## MATERIALS AND METHODS

### Genetic materials

Ten sunflower inbred lines were chosen to estimate the genetic distance among them and looking for genotype specific markers. The experiment was carried out at laboratories and greenhouse of Cell Research Department, Field Crops Research Institute, Agricultural Research Center. These inbred lines were exported from different origins as follows: $P_1$= Ha89, $P_2$= Ha93, $P_3$= Ha64, $P_4$= Ha101, $P_5$= Ha122 (USDA), $P_6$= Sha13, $P_7$= Sha14, $P_8$= Sha15 (ARC, El-Serw Agricultural Research Station) and

$P_9$= Nsha136 and $P_{10}$= Nsha140 **(**Yugoslavia). Lines were grown in pots filled with clay and peat moss. Leaves of these inbred lines were cut and put in liquid nitrogen (-196$^o$C) to be transfer to the lab.

**DNA isolation and PCR**

Total DNA was isolated from young leaves of the ten sunflower inbred lines according to modified CTAB method described by Khaled and Esh (2008). DNA concentrations and their purity were determined by taking A260 and A260/A280 values in UNICO UV-2000 Spectrophotometer (from Shanghai China). The ten sunflower inbred lines were analysed at the molecular level by using 10 pre-screened ISSR primer: HB-10, HB- 11, HB-12, HB-13, HB-14, HB-15, 14A, 1-5A, 2-5A and 3-5A and 10 pre-screened RAPD primers: OP-A08, OP-A11, OP-A15, OP-B05, OPB15, OP-B19, OP-B20, OP-C01, OP-C07 and OPC17 to achieve ISSR and RAPD fragments, as shown in Table (1).

**Table 1. List of ISSR and RAPD primers and their sequences.**

| ISSR Primer names | Sequence | RAPD Primer names | Sequence |
|---|---|---|---|
| **HB-11** | **5'-GT GT GT GT GT GCC(GT)7 GCC -3'** | **OP-A08** | **5'-GTGACGTAGG-3'** |
| **HB-13** | **5'-GAGGAGGAGGC-3'** | **OP-A11** | **5'-CAATCGCCGT-3'** |
| **HB-15** | **5'-GTGGTGGTGGC-3'** | **OP-A15** | **5'-TTCCGAACCC-3'** |
| **HB-12** | **5'-CACCACCACGC-3'** | **OP- B05** | **5'-TGCGCCCTTC-3'** |
| **HB-10** | **5'GAGAGAGAGAGACC(GA)6CC-3'** | **OP-B15** | **5'-GGAGGGTGTT-3'** |
| **HB-14** | **5'-CTCCTCCTCGC-3'** | **OP- B19** | **5'-ACCCCCGAAG-3'** |
| **14-A** | **5'-CTCTCTCTCT CT CT CTTG(CT)8TG-3'** | **OP-B20** | **5'-GGACCCTTAC-3'** |
| **1-5A** | **5'-GAG AGA GAG AGA GAG ATC-3'** | **OP-C01** | **5'-TTCGAGCCAG-3'** |
| **2-5A** | **5'-AGAGAGAGAGAGAGAGC-3'** | **OP-C07** | **5'-GTCCCGACGA-3'** |
| **3-5A** | **5'-ACACACACACACACACG-3'** | **OP- C17** | **5'-TTCCCCCCAG-3'** |

Polymerase chain reaction (PCR) was performed in Gradient Cycler (BioRAD, USA) according to Williams *et al* (1990). Reaction mixture for PCR consisted of 25µl and for ISSR primers consisted of 3 µl of 10 X Taq assay buffer, 2 µl of 2.5 mM dNTP (Promega, Madison, USA), 2 µl Taq DNA polymerase (5 unit/1µl) (Promega, Madison, USA), 50ng genomic DNA, 2 µl Primer (10 pmol), while for RAPD primers the reaction mixture for PCR (25µl) consisted of 3 µl of 10 X Taq assay buffer, 2 µl of 2.5 mM dNTP (from Promega, Madison, USA), 2 µl Taq DNA polymerase (500 unit/1µl) (from Promega, Madison, USA), 50ng genomic DNA, 2 µl Primer

(10 pmol) and 2.5 µl MgCl2 (25 mM). The amplification carried out in a DNA Thermocycler MJ 200CT. For the ISSR primers, the PCR protocol was programmed as: initial denaturation at 94ºC for 5 min, 35 cycles' 94ºC/1 min, 55ºC/1 min, 72ºC/2 min and final extension at 72ºC/10 min, while for RAPD primers the PCR protocol was programmed as: initial denaturation at 94ºC for 2 min, 45 cycles' 94ºC/1 min, 35ºC/90s, 72ºC/2 min and final extension at 72ºC/5min for RAPD primers.

**DNA electrophoresis and analysis**

Amplification product were checked on 1.50% agarose gels in 1 X TBE buffer and visualized by staining with 0.01% ethidium bromide. For comparison of molecular size of amplicons produced by ISSR primers 50bp DNA ladder were used with molecular weights of 1500, 1200,1000, 900, 800, 700, 600, 500, 450, 400, 350, 300, 250, 200, 150, 100 and 50 bp, while for comparison of molecular size of amplicons produced by RAPD primers 100 bp ladder were used with molecular weights of 3000, 1500, 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp. The runs were performed for about 30min at 90 V in Pharmacia submarine (7 cm X 7 cm).

Gels were photographed under UV light and score using gel documentation system manufactured by Alpha Ease FC (AlphimagerTm 2200), U.S.A. Only reproducible and distinct bands were scored as 1/0 (presence /absence) for data analysis. The data of RAPD and ISSR analyses were entered in a computer file as binary matrices. Similarity coefficients were calculated according to Dice matrix (Dice 1945). Genetic distances were calculated and constructions of the dendrogram trees were performed using the unweighted pair group method based on arithmetic mean (UPGMA) as implemented in the SPSS Program Version 22 (Yang and Quiros 1993).

## RESULTS AND DISCUSSION

**ISSR analysis**

**Polymorphism and molecular markers**

The results of amplified fragments by different ISSR primers are shown in Fig. (1) and Table (2). Out of ten ISSR primers, only eight produced scorable banding patterns for the ten sunflower inbred lines. A total of 95 major scorable bands ranging from 117 to 1075 bp were generated, out of which only 78 fragments were polymorphic with 78.8 % polymorphism. The number of bands per ISSR ranged from 8 (HB-10 and HB-14) to 20 (HB-13) with an average of 11.9 bands per primer. Primers HB-13 and HB-15 displayed the maximum polymorphism (100%).

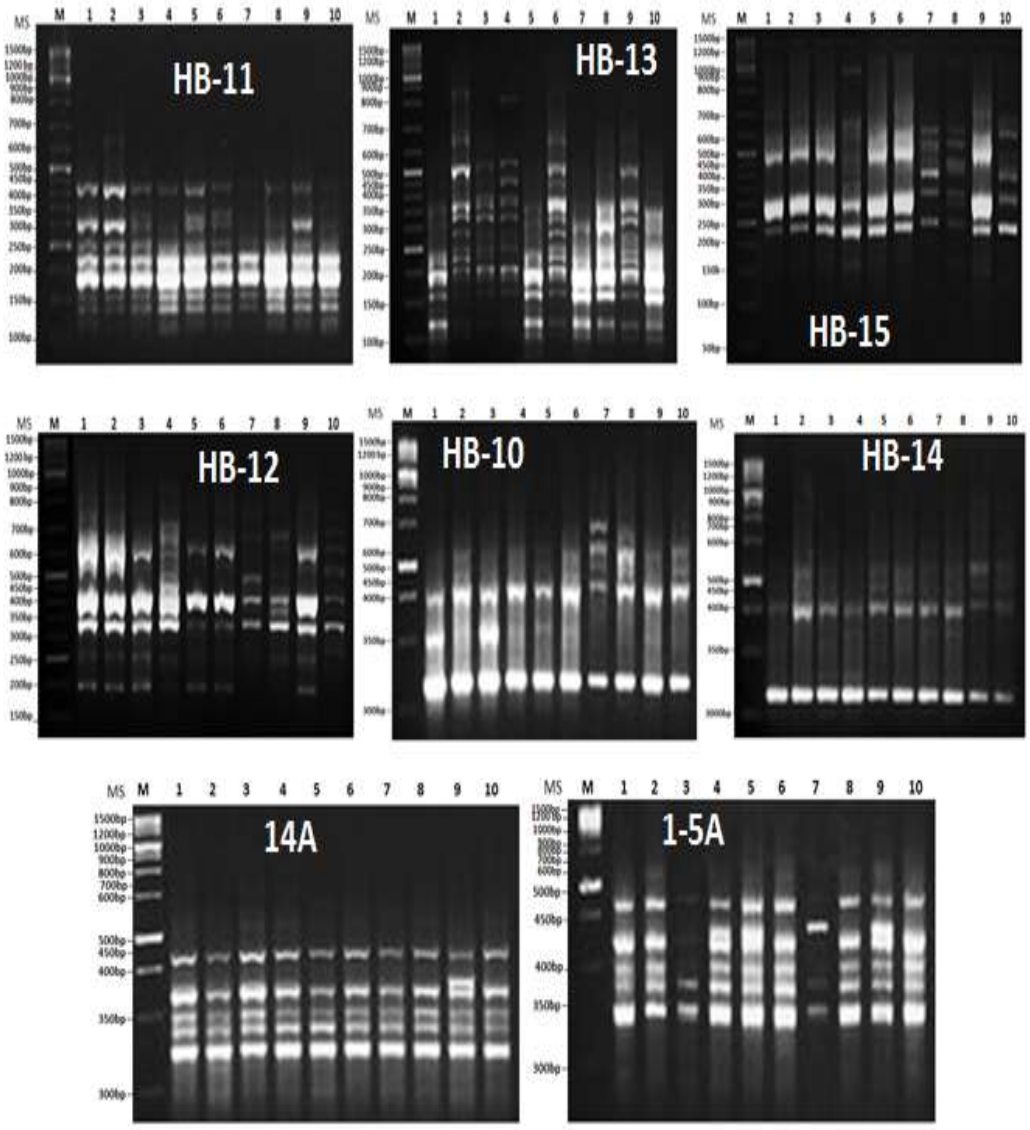


**Fig.1. ISSR amplification products from ten sunflower inbred lines using eight primers.**

**where, M= 50 bp ladder, 1=$P_1$= Ha89, 2=$P_2$= Ha93, 3=$P_3$= Ha64, 4=$P_4$= Ha101, 5=$P_5$= Ha122, 6=$P_6$= Sha13, 7=$P_7$= Sha14, 8=$P_8$= Sha15, 9=$P_9$= Nsha136 and 10= $P_{10}$=Nsha140**

**Table 2. Summary of the generated genotypes specific markers using 8 ISSR primers across ten sunflower inbred lines.**

| Primer type | Primer name | MS Bp | Total bands | MMB | PMB | Polymorphism % | Genotype specific marker |
|---|---|---|---|---|---|---|---|
| ISSR | HB-11 | 122 – 608 | 11 | 4 | 7 | 63.6 | $P_2$ at 608, $P_6$ at 341 and $P_5$ |
| | HB-13 | 117 – 927 | 20 | 0 | 20 | 100 | $P_4$ at 538, $P_2$ at 437, $P_1$ at |
| | HB-15 | 151 - 1075 | 14 | 0 | 14 | 100 | $P_4$ at 1075, $P_4$ at 203 and $P_9$ |
| | HB-12 | 200 – 940 | 13 | 2 | 11 | 84.6 | $P_4$ at 940, $P_4$ at 748 and $P_{10}$ |
| | HB-10 | 165 – 697 | 8 | 2 | 6 | 75 | $P_7$ at 697, $P_8$ at 627 and $P_7$ at |
| | HB-14 | 265- 619 | 8 | 2 | 6 | 75 | $P_2$ at 619 and $P_3$ at 265 bp |
| | 14A | 130 – 417 | 10 | 5 | 5 | 50 | $P_1$ at 364 and $P_9$ at 338 bp |
| | 1-5A | 426 – 939 | 11 | 2 | 9 | 81.8 | $P_6$ at 454 and 426 bp |
| | Total | | 95 | 17 | 78 | | 22 |
| | Mean | | 11.9 | 2.1 | 9.8 | 78.8 | 2.8 |

**MMB = Monomorphic band PMB = Polymorphic band MS = Molecular size**

This makes them highly heterogeneous and consequently appears broad genetic variation among them. While, the other ISSR primers detected more than 70% polymorphism except the primers 14A and HB-11 showed 50 and 63.6% polymorphism, respectively. All ISSR primers produced unique bands (genotype specific markers) that could be used for distinguish among the inbred lines. Maximum number of ISSR unique bands were 4 that produced by Primer HB-13. Garayalde *et al* (2011) found 9 ISSR private bands in wild accessions of sunflower.

**Genetic similarity and cluster analysis based on ISSR data**

The highest similarity value 84.1% was observed between $P_9$ and $P_6$ inbred lines, which indicates that these inbred lines are closely related to each other. However the lowest similarity value was 51.1% that recorded between $P_7$ and $P_2$ confirming that these two inbred lines are relative dissimilar. Similarity indices of the ten sunflower inbred lines based on ISSR data are presented in Table (3). The dendrogram of the ten sunflower inbred lines showed that they were mainly separated into two main clusters at a similarity coefficient of 0.25. Inbred lines $P_7$, $P_8$ and $P_{10}$ formed the first cluster. While, the second cluster had all remaining sunflower inbred lines, which was further divided into two sub-clusters, one of them included $P_4$ alone, while the other sub-cluster was included the rest of the inbred lines, which is consequently divided into many other sub-sub-clusters, the first one gathered $P_2$ and $P_3$ together, while the other contained $P_6$, $P_9$, $P_5$ and $P_1$.

These inbred lines divided in another sub- sub-sub cluster, ended by gathering $P_6$ and $P_9$ in the same group, as shown in Fig. (2). These results are similar with those obtained by Yang *et al* (2012) and Shehata *et al* (2014).

**Table 3. Similarity indices (Pairwise comparison) of the ten sunflower inbred lines based on ISSR data.**

| Inbred lines | $P_1$ | $P_2$ | $P_3$ | $P_4$ | $P_5$ | $P_6$ | $P_7$ | $P_8$ | $P_9$ |
|---|---|---|---|---|---|---|---|---|---|
| $P_2$ | 72.9 | | | | | | | | |
| $P_3$ | 72.1 | 81.3 | | | | | | | |
| $P_4$ | 64.6 | 67.9 | 70.7 | | | | | | |
| $P_5$ | 78.7 | 70.7 | 74.2 | 62.7 | | | | | |
| $P_6$ | 72.9 | 81.1 | 75.0 | 69.7 | 76.8 | | | | |
| $P_7$ | 52.4 | 51.1 | 52.4 | 53.6 | 59.8 | 57.4 | | | |
| $P_8$ | 66.0 | 63.5 | 59.6 | 72.9 | 70.1 | 71.2 | 80.4 | | |
| $P_9$ | 80.4 | 78.5 | 78.4 | 69.1 | 82.0 | 84.1 | 58.9 | 74.3 | |
| $P_{10}$ | 69.7 | 68.7 | 62.9 | 68.6 | 73.9 | 72.7 | 73.6 | 80.4 | 72.0 |

**$P_1$= Ha89, $P_2$= Ha93, $P_3$= Ha64, $P_4$= Ha101, $P_5$= Ha122, $P_6$= Sha13, $P_7$= Sha14, $P_8$= Sha15, $P_9$= Nsha136 and $P_{10}$= Nsha140**

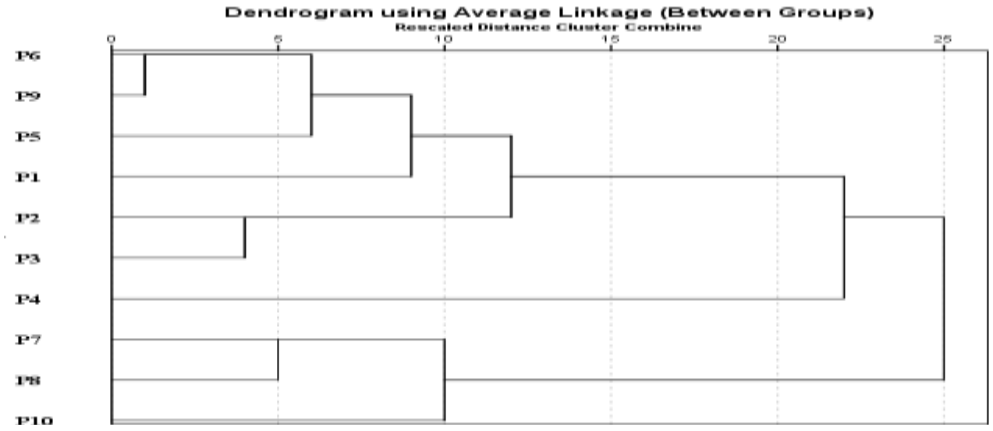


**Fig. 2. UPGMA clustering dendrogram illustrates the genetic relationship among ten sunflower inbred lines based on ISSR data.**

**$P_1$= Ha89, $P_2$= Ha93, $P_3$= Ha64, $P_4$= Ha101, $P_5$= Ha122, $P_6$= Sha13, $P_7$= Sha14, $P_8$= Sha15, $P_9$= Nsha136 and $P_{10}$= Nsha140.**

**RAPD analysis**

**Polymorphism and molecular markers**

The results of amplified fragments by different RAPD primers are shown in Fig. (3) and Table (4).

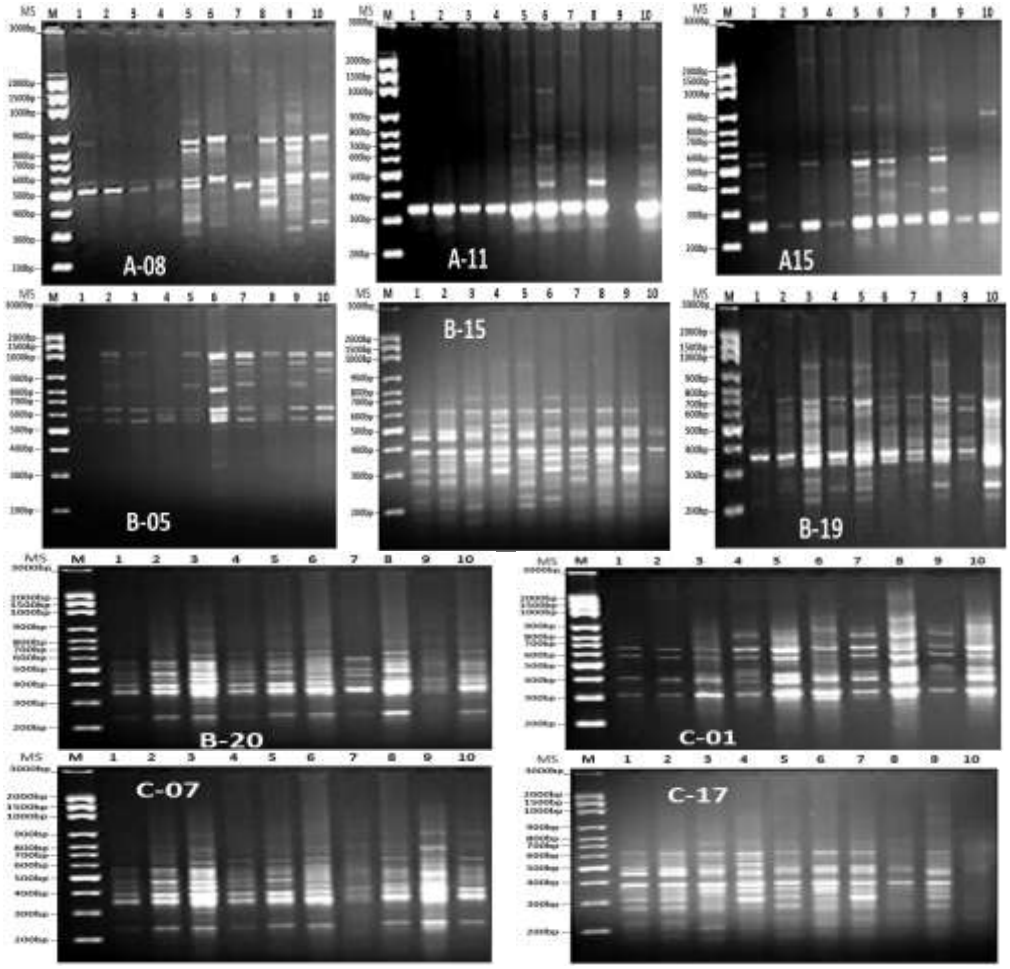


**Fig. 3. RAPD amplification products generated from ten sunflower inbred lines using ten primers.**

**where, M= 100 bp ladder, 1=$P_1$= Ha89, 2=$P_2$= Ha93, 3=$P_3$= Ha64, 4=$P_4$= Ha101, 5=$P_5$= Ha122, 6=$P_6$= Sha13, 7=$P_7$= Sha14, 8=$P_8$= Sha15, 9=$P_9$= Nsha136 and 10= $P_{10}$=Nsha140.**

**Table 4. Summary of the generated genotypes specific markers using 10 RAPD primers across ten sunflower inbred lines.**

| Primer type | Primer name | MS bp | Total bands | MMB | PMB | Polymorphism % | Genotype specific marker |
|---|---|---|---|---|---|---|---|
| RAPD | A-08 | 323- 1972 | 21 | 0 | 21 | 100 | $P_9$ at 1690, 1548, 1381, 1226, 742 and $P_5$ at 735 bp |
| | A-11 | 246- 2028 | 10 | 0 | 10 | 100 | - |
| | A15 | 248 -2835 | 14 | 1 | 13 | 92.8 | $P_{10}$ at 976 , $P_5$ at 437 , $P_7$ at 417, $P_8$ at 382 and $P_6$ at 337 bp |
| | B-05 | 29- 1410 | 12 | 2 | 10 | 83.3 | $P_6$ at 994, 810, 484 and 290 bp |
| | B-15 | 129 -1177 | 19 | 1 | 18 | 94.7 | $P_6$ at 389 bp |
| | B-19 | 169 -1981 | 16 | 0 | 16 | 100 | $P_7$ at 1981, $P_6$ at 1015 and $P_5$ at 250 bp |
| | B-20 | 204- 976 | 15 | 12 | 13 | 86.7 | $P_3$ at 976, $P_{10}$ at 420, $P_3$ at 259 and $P_7$ at 204 bp |
| | C-01 | 230- 1165 | 12 | 3 | 9 | 75 | $P_5$ at 774 bp |
| | C-07 | 202 – 978 | 15 | 2 | 13 | 86.7 | $P_8$ at 420 bp |
| | C-17 | 133 -625 | 16 | 1 | 15 | 93.8 | $P_7$ at 702 bp |
| | Total | | 150 | 22 | 138 | | 26 |
| | Mean | | 15 | 2.2 | 13.8 | 91.3 | 2.6 |

**MMB= Monomorphic band PMB= Polymorphic band MS=Molecular size**

Ten RAPD primers produced scorable banding patterns for the ten sunflower inbred lines. The molecular size of the amplified DNA bands ranged from 129 to 2835 bp. Highest number of bands (21) were generated by OP-A08, whereas A-11 produced the lowest number of bands were 10. A total of 150 amplified fragments (loci) were produced. Out of them 138 were polymorphic with 91.3% polymorphism. Raza *et al* (2018) observed 86.34% polymorphic bands by using 20 RAPD primers in 10 sunflower genotypes. The highest percentage of polymorphism was 100 %, which scored by primers OPA-08, OPA-11 and OPB-19. While, primer OPC-01 produced lowest polymorphism percentage was 75%. All RAPD primers produced unique bands that could be used for distinguish among the inbred lines except primer OPA-11. Maximum number of unique bands was 6, which was produced by primer OPA-08.Genetic similarity and cluster analysis based on RAPD data

The RAPD data were used to estimate the genetic similarity values among ten sunflower inbred lines Table (5).The highest similarity value between the ten sunflower inbred lines was 82.4% which recorded between Ha89 and Ha93 inbreds. However, the lowest similarity value was 38.9% which observed between Ha89 and Nsha140 inbreds. Also, Popov *et al* (2002) and Isaacs *et al* (2003) found high level of genetic diversity of RAPD markers in sunflower inbred lines.

**Table 5. Similarity indices (Pairwise comparison) of the ten sunflower inbred lines based on RAPD data.**

| Inbred lines | $P_1$ | $P_2$ | $P_3$ | $P_4$ | $P_5$ | $P_6$ | $P_7$ | $P_8$ | $P_9$ |
|---|---|---|---|---|---|---|---|---|---|
| $P_2$ | 82.4 | | | | | | | | |
| $P_3$ | 61.8 | 70.2 | | | | | | | |
| $P_4$ | 58.5 | 63.2 | 69.6 | | | | | | |
| $P_5$ | 58.6 | 60.8 | 67.5 | 64.5 | | | | | |
| $P_6$ | 50.0 | 58.1 | 62.7 | 60.5 | 73.1 | | | | |
| $P_7$ | 50.0 | 61.7 | 62.4 | 61.3 | 63.3 | 59.5 | | | |
| $P_8$ | 53.7 | 59.5 | 64.5 | 57.8 | 68.6 | 66.3 | 59.6 | | |
| $P_9$ | 50.0 | 58.1 | 55.2 | 50.0 | 55.6 | 58.0 | 59.7 | 59.3 | |
| $P_{10}$ | 38.9 | 46.6 | 52.6 | 45.0 | 58.4 | 58.4 | 47.6 | 68.6 | 46.2 |

**$P_1$= Ha89, $P_2$= Ha93, $P_3$= Ha64, $P_4$= Ha101, $P_5$= Ha122, $P_6$= Sha13, $P_7$= Sha14, $P_8$= Sha15, $P_9$= Nsha136 and $P_{10}$= Nsha140**

The dendrogram related to RAPD data is shown in Fig. (4). The dendrogram of ten inbred lines of sunflower showed that they mainly separated into three main clusters at a similarity coefficient of 0.25. Sunflower inbred line Nsha136 was out-grouped from all other inbreds and formed the first cluster. As well as, Sha15 and Nsha140 were located together in the second cluster out-grouped. The third cluster had all remaining inbreds, which was further divided into two sub-clusters at a similarity coefficient of 0.21. The first was further divided into two sub-sub-clusters, the first one contained Ha89 and Ha93 and the second was divided into two sub-sub-clusters the first one contained only Sha14 and the second one separated the remaining inbreds of sunflower to two groups Ha122 and Sha13 together and Ha64 and Ha101 together. There are many previous researchers used RAPD technique in sunflower among them Lawson *et al* (1994); Arias and Rieseberg (1995); Atanasova *et al* (2004); Nandini and Chikkadevaiah (2005); Mohan and Seetharam (2005); Saftić-Panković (2007) and Iqbal *et al* (2008).

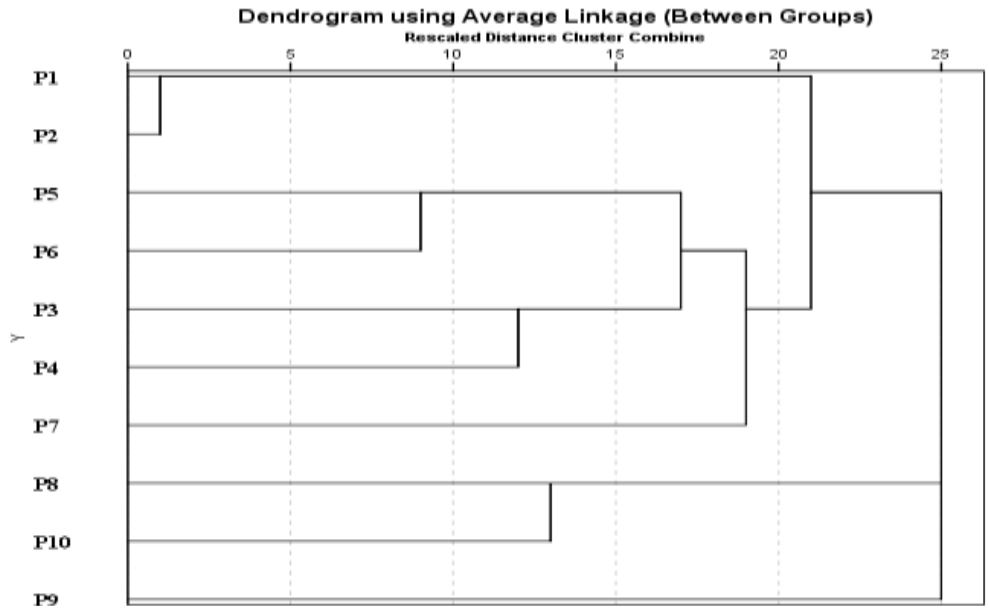


**Fig.4. UPGMA clustering dendrograms illustrates the genetic relationship among ten sunflower inbreds based on RAPD data.**

**$P_1$= Ha89, $P_2$= Ha93, $P_3$= Ha64, $P_4$= Ha101, $P_5$= Ha122, $P_6$= Sha13, $P_7$= Sha14, $P_8$= Sha15, $P_9$= Nsha136 and $P_{10}$= Nsha140.**

Tables (2) and (4) summarize the total bands (monomorphic and polymorphic), polymorphism percentage and revealed genotype specific markers generated by the eight ISSR and the ten RAPD primers in ten sunflower inbred lines. The mean of bands per ISSR primer was 15 higher than the mean of band per RAPD primer, which were 11.9. On the contrary, the mean of polymorphism percentage for RAPD markers was 91.3 higher than the mean of polymorphism percentage for ISSR markers (78.8) revealing the high precision and discrimination ability of both techniques, especially RAPD marker technique in wider context. These results are in harmony with Mahmoud and Abdel-Fatah (2012) and Karande (2015) who reported that the polymorphism percentage over RAPD markers was higher than ISSR markers in sunflower, but Kumar *et al* (2017) found that ISSR polymorphism % was higher than the polymorphism of RAPD in sunflower. In this study, the mean of number of genotype specific markers per ISSR primers was 2.8. While the mean number of genotype specific markers per RAPD primers was 2.6.

**Genetic similarity and cluster analysis based on combined data**

The combined data of RAPD and ISSR were used to estimate the genetic similarity values among ten sunflower inbreds Table (6). The highest similarity value between the ten sunflower inbred lines 77.8% was recorded between Ha89 and Ha93 inbreds. However the lowest similarity value was 51% and was observed between Ha89 and Sha14 inbreds.

**Table 6. Similarity indices (Pairwise comparison) of the ten sunflower inbred lines based on ISSR and RAPD combined data.**

| Inbred lines | $P_1$ | $P_2$ | $P_3$ | $P_4$ | $P_5$ | $P_6$ | $P_7$ | $P_8$ | $P_9$ |
|---|---|---|---|---|---|---|---|---|---|
| $P_2$ | 77.8 | | | | | | | | |
| $P_3$ | 66.0 | 74.9 | | | | | | | |
| $P_4$ | 61.5 | 65.5 | 70.1 | | | | | | |
| $P_5$ | 66.4 | 64.8 | 69.8 | 63.8 | | | | | |
| $P_6$ | 59.3 | 67.7 | 67.2 | 64.4 | 74.4 | | | | |
| $P_7$ | 51.0 | 57.0 | 58.7 | 57.9 | 62.0 | 58.7 | | | |
| $P_8$ | 59.0 | 61.3 | 62.6 | 64.5 | 69.2 | 68.1 | 67.8 | | |
| $P_9$ | 63.8 | 67.5 | 64.5 | 58.8 | 65.6 | 68.4 | 59.4 | 65.6 | |
| $P_{10}$ | 52.8 | 56.7 | 56.6 | 55.9 | 64.2 | 64.0 | 58.2 | 73.5 | 57.4 |

**$P_1$= Ha89, $P_2$= Ha93, $P_3$= Ha64, $P_4$= Ha101, $P_5$= Ha122, $P_6$= Sha13, $P_7$= Sha14, $P_8$= Sha15, $P_9$= Nsha136 and $P_{10}$= Nsha140**

The dendrogram related to ISSR and RAPD combined data is shown in Fig. (5). The dendrogram of the ten sunflower inbred lines showed that they were mainly separated into two main clusters at a similarity coefficient of 0.25. The first cluster included Sha14, Sha15 and Nsha140 that were out-grouped from all other inbreds. The second cluster had all remaining inbreds, which was further divided into two sub-clusters at a similarity coefficient of 0.18. The second sub cluster was further divided into two sub-sub-clusters the first one contained Nsha136 alone, while the other contained Ha122 and Sha13 together. On the other hand, the second sub-sub-clusters was further divided into two groups, the first contained Ha101 alone, while, the second contained Ha64, Ha93 and Ha89 together. These results are in agreement with Azzam (2005) and Azzam and Abo-Doma (2007), who observed some genetic relationships among some sunflower and canola cultivars, respectively based on ISSR and RAPD data. Also, there are many investigators who studied the genetic diversity of sunflower and other oil crops based on ISSR and RAPD molecular markers, among them Joshi *et al*. (2004); Valdemar *et al*. (2004); Azzam *et al* (2007), Abd

El-Hadi *et al* (2008), Abdel-Tawab *et al* (2008), Golkar *et al* (2011), Idris *et al* (2012), El-Hosary and El-Akkad (2015), Houmanat *et al* (2016), Azzam and Khalifa (2016), Muhammad *et al* (2017), Safavi *et al* (2017) and Abbas *et al* (2019) **.**

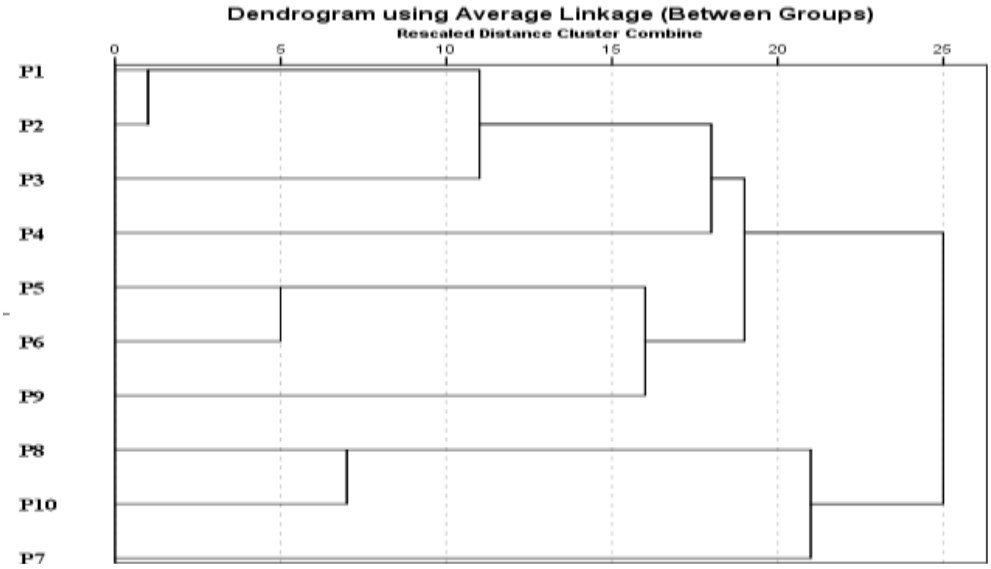


**Fig. 5. UPGMA clustering dendrograms illustrates the genetic relationship among ten sunflower inbred lines based on RAPD and ISSR combined data.**

**$P_1$= Ha89, $P_2$= Ha93, $P_3$= Ha64, $P_4$= Ha101, $P_5$= Ha122, $P_6$= Sha13, $P_7$= Sha14, $P_8$= Sha15, $P_9$= Nsha136 and $P_{10}$= Nsha140.**

The results of present study confirms that ISSR and RAPD marker are efficient in detecting polymorphism and genotype specific markers among sunflower inbred lines. As a general conclusion, our investigation revealed that ISSR technique is better than RAPD technique to obtain genotype specific markers in sunflower, but, RAPD technique is better than ISSR technique in order to get the highest percentage of polymorphism. ISSR and RAPD also determining the contrasts parents to gain heterosis and successful sunflower breeding programs. Results also demonstrated that parents who showed the lowest genetic similarity values among each other might be display the highest significant heterosis values in their crosses for most studied traits, especially yield and yield components.

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# العلاقات الوراثية بين عشرة سلالات تربية ذاتية من زهرة الشمس اعتمادا علي تحليلات ISSR و RAPD

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***تم إجراء هذا البحث من أجل معرفة التنوع الوراثي بين عشرة سلالات تربية ذاتية من زهرة الشمس والتي تم الحصول عليها من مصادر مختلفة وهم : Ha89، Ha93، Ha64، Ha101، Ha122، Sha13، Sha14، Sha15، Nsha136 و Nsha140 وذلك باستخدام تقنيات ISSR و RAPD. تم الحصول علي ٩٥ من الحزم الرئيسة بواسطة ٨ بادئات الـ ISSR يتراوح وزنها الجزيئي من ١١٧ إلي ١٠٧٥ زوج من القواعد وأظهرت ٧٨,٨٪ تنوع جزيئي. بينما بادئات RAPD العشرة أنتجت ١٥٠من الحزم تراوح وزنها الجزيئي من١٢٩ إلي٢٨٣٥ زوج من القواعد وكشفت عن تنوع جزيئي مقداره ٩١,٣٪ انتج كلا من ISSRs و RAPDs واسمات جزيئية متفردة. وقد تراوحت قيم التشابه الوراثي بين سلالات زهرة الشمس ما بين ٥١,١-٨٤,١٪ و ٣٨,٩-٨٢,٤٪ للـ ISSR, RAPD علي التوالي. وقد لوحظ أن أقل تشابه باستخدام بادئات ISSR بين السلالاتين Sha14 و Ha93، بينما كان باستخدام بادئات الـ RAPD بين السلالتين Ha89 و Nsha140. وقد قام الـ dendrogram المعتمد علي بيانات الـ ISSR بتقسيم العشرة سلالات إلي مجموعتين رئيسيتين عند معامل تشابه ٠,٢٥. بينما قسمهم الـ dendrogram العائد إلي بيانات الـ RAPD إلي ثلاث مجموعات رئيسية. البيانات المجمعة لل ISSR، ال RAPD معا أظهرتا تباين وراثي يتراوح من ٥١,٠ إلي ٧٧,٨٪. كذلك أعطي الـ dendrogram الراجع إلي البيانات المجمعة لكلا من الـ ISSR، RAPD نتائج مشابهة إلي حدا كبير لتلك التي تم الحصول عليها من تحليل ISSR الفردي.***

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