Egypt. Acad. J. Biolog. Sci., 12(1):65-81 (2020)

Egyptian Academic Journal of Biological Sciences

C. Physiology & Molecular Biology

ISSN 2090-0767

www.eajbsc.journals.ekb.eg

Phylogenetic Relationships between Ten Genotype of *Hordeum vulgare* L. Using Molecular Markers

H. El-Atroush[1*], Z. M. El-Ashry[2] and Samira A. Osman[2]

1- Botany Department, Faculty of Science, Ain Shams University, Abassia, Cairo, Egypt.

2- Genetics and Cytology Department, National Research Centre, Giza, Egypt.

E.Mail: hala_elatroush@hotmail.com

ARTICLE INFO

Article History

Received:15/2/2020

Accepted:14/3/2020

Keywords:

Hordeum vulgare L, phylogenetic relationships, RAPD, ISSR, molecular markers.

ABSTRACT

Phylogenetic relationships between ten *Hordeum vulgare* L. , seven cultivars (Giza 123, Giza 126, Giza 127, Giza 128, Giza 129, Giza 130 and Giza 2000) and three landraces from Sinai (El-Kheroba, El-Sheikh Zuwaid, and Wadi Sedr) were carried out using two molecular genetic markers (RAPD-PCR and ISSR-PCR). The genetic distance between Ten genotype was also estimated from banding patterns Twenty two random primers were used in RAPD revealed 316 bands while 159 bands were detected of ISSR analysis using 10 primers. RAPD analysis among ten genotypes showed 41.77% polymorphism, while ISSR analysis showed 62.02% polymorphism.

It was found that, ISSR was a more viable marker than RAPD in the detection of the genetic variability among *Hordeum vulgare* (barley) varieties. The genetic distance tree was detected using UPGMA based on both molecular markers (RAPD and ISSR) and analysis of combined data.In addition, the Band Shearing index (BSI) factor was calculated shows a marked difference between the ten genotypes, seven cultivars and three landraces of studied *Hordeum vulgare* where BSI average reach 1.42% with RAPD markers while reaching 0.93 % in ISSR. The obtained data indicated that both RAPD and ISSR markers are efficient in identification and differentiation between selected taxa, but the efficiency of ISSR was the best one. Also, the present results, enhancing the available knowledge of *Hordeum vulgare* genetic resources in Egypt, which may contribute to their conservation and utilization in breeding programs.

INTRODUCTION

The genus *Hordeum* L. includes 30 species of annual and perennial grasses; the cultivated barley (*Hordeum vulgare* L.) is one of the main cereals of the belt of the Mediterranean agriculture and a founder crop of Old World Neolithic food production (Harlan and Zohary, 1966). phylogenetic relationships is important in the conservation and restoration of biodiversity of wild germplasm (Wang *et al*., 2009).

Recently, genetic markers, which evaluate diversity at the molecular level, were proposed to assess genetic variability. DNA based molecular markers help plant breeders to directly evaluate genetic variation among relatives without the effect of environmental factors (Nguyen *et al*., 2004). Several molecular markers were used to evaluate the extent of genetic variability, such include Random Amplified Polymorphic DNA (RAPD) (Yu *et al*., 2002 and Mylonas *et al*., 2014), Inter Simple Sequence Repeats (ISSR) (Brantestam *et al*., 2004; El-Atroush *et al*., 2015; Goyat *et al*., 2016 and Sharma *et al*., 2016).

Random amplified polymorphic DNA (RAPD) markers (Williams *et al.*, 1990) have advantages including speed, low cost, independent from developmental stages of the plants and require only small amounts of sample (Bardakci, 2001). Although RAPDs are dominant markers, they have been used for genome mapping, identifying cultivars, breeding and analyzing genetic variation within and among populations (both intra- and interspecies) (Tsuda *et al.*, 2004). Inter-simple sequence repeat (ISSR) technique is useful in studying genetic diversity and DNA fingerprinting (Martin and Sanchez-Yelamo, 2000) especially in cultivated plants including barley (Fernandez *et al.*, 2002). ISSR markers are advantageous in view of the production of considerable numbers of fragments per primer at relatively low cost and their reproducibility (Moreno *et al.*, 1998 and Salimath *et al.*, 1995).

The aim of the present study is to assess the phylogenetic relationships between ten Egyptian *Hordeum vulgare* L varieties depending on molecular genetic markers RAPD and ISSR, also the determination of the viable marker which has potential power in discrimination and identification of cultivars and unknown landraces which present in this study.

MATERAILS AND METHODS

Seeds of seven cultivars of *Hordeum vulgare* L. (Giza 123, Giza 126, Giza 127, Giza 128, Giza 129, Giza 130 and Giza 2000) were obtained from Agricultural Research Center, Giza, Egypt. While three landraces varieties were obtained from Egyptian National Gen Bank, Agricultural Research Center, Giza, Egypt, under code number (11557, 11580 and 113737). These landraces collected from Sinai, El-Aresh (El-Kheroba, El-Sheikh Zuwaid) and Ras Sedr (Wadi Sedr) as shown in Table (1).

Table 1: The pedigree of studied *Hordeum vulgare L.*genotype.

No.	Accessions	Pedigree	Origin
1	Giza 123	Giza 117 / FAO86.	Egypt
2	Giza 126	Baladi Bahteem/S D729-Por12762-BC.	Egypt
3	Giza 127	W12291/Bags // Harmal- 02.	Egypt
4	Giza 128	*WI2291 / 4 /11012-270-22425 / 3 /Apam / IB65 //A 16.*	Egypt
5	Giza 129	DeirAlla 106/Cel//AS 46/Aths*2.	Egypt
6	Giza 130	*Comp.cross 229 // Bce Mr /DZ 02391 / 3 / Deir 106A lla106.*	Egypt
7	Giza 2000	Giza117/Bahteem52// Giza118/ FAO86 / 3/ Baladi16/ Gem.	Egypt
8	Landraces	Sinai, El-Aresh, El-Kheroba (Egy., GenBank, code No. 11557).	Egypt
9	Landraces	Sinai, El-Aresh, El-Sheikh Zuwaid (Egy., GenBank, code No. 11580).	Egypt
10	Landraces	Sinai, Ras Sedr, Wadi Sedr (Egy., GenBank, code No. 113737).	Egypt

Molecular Studies :

DNA was isolated from ten studied *Hordeum vulgare* L. varieties by using thermo-DNA extraction kits (Fermentus). 100 mg of plant leaves were grinded in the seedling stage (2-3 weak old plant) with liquid nitrogen, then 350 µl of Lysis Buffer A was added then it was mixed thoroughly by vortex for 10-20 seconds. 50 µl of Lysis Buffer B and 20 µl RNase-A were added. The sample was Incubated 10 min at 65°C while vortexing occasionally, then 130 µl Precipitation Solution was added and mix by inverting the tube 2-3 times. The sample was Incubated 5 min on ice, then was centrifuged for 5 min at 14000 rpm. The supernatant (usually 450-550 µl) was collected and was transferred to the clean microcentrifuge tube then 400 µl of Plant gDNA Binding Solution and 400µl of 96% ethanol was added and were mixed well. Half of the prepared mixture was transferred to the spin column and centrifuged for 1 min at 8000 rpm. The flow was discarded through the solution and

the remaining mixture was applied onto the same column and was centrifuged for 1 min at 8000 rpm then 500 µl of Wash Buffer I and centrifuge were added for 1 min at 10000 rpm then the flow was discarded and the column was placed back into the collection tube then 500 µl of Wash Buffer II was added to the column and was centrifuged for 3 min at maximum speed 14000 rpm. The purification column was placed back into the tube and was re-spined the column for 1 min. at maximum speed 14000 rpm then the collection tube was discarded containing the flow-through solution and the column was transferred to a sterile 1.5 ml micro-centrifuge tube and 100 µl of Elution Buffer was added to the centre of the column membrane to elute genomic DNA. The purified DNA is ready to be used or, be stored at -20°C.

Random Amplified Polymorphic DNA (RAPD-DNA):

Twenty-two RAPD primers were used as showed in Table (2). PCR amplification for different isolated DNA was performed in 0.2 ml PCR ppendorf containing (50 µl) consisted of Dream *Taq* DNA Polymerase, Fermentus (1 µl), 10x Dream *Taq* Green Buffer (5µl), dNTP Mix 10mM each (1µl), primer, Metabion, German (2 µl) and Template DNA (2 µl) then completed to 50µl by Water, nuclease-free. Thermocycler (Bio-Rad) was programmed for 35 cycles as follows: 94°C for 5 min (one cycle) then 94°C for 1 min, Tm°C for 45 sec and 72°C for 45 sec (35 cycles) then 72°C for 5 min (one cycle) then held at 4°C.

Table 2: List of RAPD primers and their nucleotide sequences.

Primer	Sequence (5'-3')	Tm	Primer	Sequence (5'-3')	Tm
RAPD1	GGA CGG CGT T	37°C	**OP-B09**	TGG GGG ACT C	37°C
RAPD2	AAC GCG CAA C	37°C	**OP-B10**	CTG CTG GGA C	37°C
RAPD3	CCC GTC AGC A	37°C	**OP-B11**	GTA GAC CCG T	37°C
RAPD4	CCA CAG CAG T	37°C	**OP-B20**	GGA CCC TTA C	37°C
RAPD5	AAG CCC GAG G	37°C	**OP-C06**	GAA CGG ACT C	38°C
OP-A01	CAG GCC CTT C	37°C	**OP-C16**	CAC ACT CCA G	38°C
OP-A11	CAA TCG CCG T	37°C	**OP-C18**	TGA GTG GGT G	38°C
OP-A16	AGC CAG CGA A	38°C	**OP-D11**	AGC GCC ATT G	38°C
OP-B03	CAT CCC CCT G	37°C	**OB-D13**	GTC AGA GTC C	37°C
OP-B05	TGC GCC CTT C	37°C	**OP-H07**	CTG CAT CGT G	37°C
OP-B08	GTC CAC ACG G	37°C	**OP-H18**	GAA TCG GCC A	37°C

Inter Simple Sequence Repeats of DNA (ISSR-DNA):

Ten ISSR primers were used as showed in Table (3). PCR amplification for different isolated DNA was performed in 0.2 ml PCR eppendorf containing (50 µl) consisted of Dream *Taq* DNA Polymerase, Fermentus (1 µl), 10x Dream *Taq* Green Buffer (5µl), dNTP Mix 10mM each (1µl), primer, Metabion, German (2 µl) and Template DNA (2 µl) then completed to 50µl by Water, nuclease-free. Thermocycler (Bio-Rad) was programmed for 35 cycles as follows: 94°C for 1 min (one cycle) then 94°C for 1 min, Tm°C for 30 sec and 72°C for 1 min (35 cycles) then 72°C for 5 min (one cycle) then held at 4°C.

Table 3: List of ISSR-DNA primers and their nucleotide sequences.

Primer	Sequence (5'-3')	Tm	Primer	Sequence (5'-3')	Tm
ISSR-1	GAG (CAA)5	49°C	ISSR-12	(AG)8YT	55°C
ISSR-3	CTG (AG)8	55°C	ISSR-14	(GA)8YC	53°C
ISSR-4	(CT)8G	52°C	C-2	(AG)8YC	55°C
ISSR-8	DBDA(CA)7	52°C	17889B	CAC ACA CAC ACA GT	48°C
ISSR-11	(AG)8	52°C	17898BS5	CACACACACACAGT	48°C

B=T or G , D=A or T or G, Y=G/C,

Agarose was placed in 1X TAE buffer (1% and 1.5% agrose gel concentration in case of RAPD and ISSR, respectively) then boiled in a water bath. Ethidium bromide was added to the melted gel after the temperature becomes 55°C. The melted gel was poured in the tray of midi-gel apparatus (horizontal electrophoresis apparatus manufactured by Cleaver, UK) and the comb was inserted immediately, then the comb was removed when the gel becomes hardened. The electrophoresis buffer (1X TAE) was added and covered the gel. 5 µl of DNA amplified product was loaded in each well and run at 100 V for about 2 hours. The gel was photographed by gel documentation (Bio-Rad) and was analyzed by the Total Lab program. to find out the molecular weight of each band and that to compare the presence and absence of the band among varieties and these dates were imported in MVSP (Multi-Variant Statistical Package) to find the similarity matrix and dendrogram (UPGMA, suing Jaccard,s coefficient) which reflect the relationships among the studied varieties.

Gene diversity referred to as Polymorphic information content (PIC) values were calculated with the following formula (Anderson *et al*., 1993):

$$PICi = 1 - \sum_{f=1}^{n} (Pij)^2$$

Where, n is the number of marker alleles for marker i and Pij is the frequency of the jth allele for marker i.

The summary statistics including the number of alleles per locus, major allele frequency, gene diversity, polymorphism information content (PIC) values were determined using Power Marker version 3.25 (Liu and Muse, 2005).

The band sharing index (BSI) was also calculated as a measure of similarity between two species using the following equation: BSI = 2S/ (C + D); where S is the number of bands shared between two samples, C is the number of bands in the first samples and D the number of bands in the second samples. A BSI value of one indicates that two species are identical, while BSI value of zero denotes that two species are totally different (Savva, 2000).

RESULTS

Polymorphism As Detected By RAPD Analysis:

In this study, a total of 316 bands were amplified among these varieties including 184 monomorphic DNA fragments and 132 were polymorphic. The polymorphic information content (PIC) varied from 0.043 to 0.204 as primer OB-D13 had the highest PIC values, while primer OP-A01 has the lowest PIC value (Table 4 and Figs. 1-3). Results of 22 RAPD primers shown 31 unique markers including 12 positive unique markers (PUM) and 19 negative unique markers (NUB). PUMs were characterized by 8 primers from 22 RAPD primers while negative unique markers (NUMs) were recorded by 10 primers from 22 RAPD primers used in this investigation. The value of the Band Sharing Index (BSI) of genotypes, cultivars, and landraces was calculated in presence of RAPD marker and the results showed that BSI has a higher percentage (1.42%) as compared with that was recorded in ISSR (0.93).; indicating more DNA variation appears with the use of ISSR (Tables 4 & 5).

Table 4: Total number of bands, polymorphic bands, percentage of polymorphism, PIC and allele size range as revealed by RAPD analysis.

Primer name	Total number of bands	No. of Polymor-phic bands	No. of Monomor-phic bands	Polymor-phism percentage	No. of Positive unique bands	No. of Negative unique bands	Allele size range (bp.)	PIC	BsI
RAPD-1	15	9	6	60%	3	-	790 - 3400	0.149	0.80
RAPD-2	16	10	6	62.5%	2	1	380 - 3370	0.17	0.75
RAPD-3	15	6	9	40%	-	-	350 - 3200	0.14	1.2
RAPD-4	13	5	8	38.46%	-	1	280 - 3300	0.116	1.23
RAPD-5	15	7	8	46.66%	1	-	320 - 3180	0.142	1.07
OP-A01	14	2	12	14.28%	-	-	135 – 2580	0.043	1.38
OP-A11	13	4	9	30.77%	-	-	160 - 2400	0.096	1.33
OP-A16	18	6	12	33.33%	1	-	220 - 3200	0.101	1.38
OP-B03	12	8	4	66.66%	1	3	500 - 3000	0.168	0.67
OP-B05	14	3	11	21.42%	1	-	250 – 3950	0.064	7.33
OP-B08	13	5	8	38.46%	-	2	350 – 3150	0.109	1.23
OP-B09	17	4	13	23.53%	-	-	780 – 4450	0.081	1.53
OP-B10	13	7	6	53.85%	-	-	180 – 2880	0.185	0.92
OP-B11	17	8	9	47.06%	1	3	350 – 4000	0.109	1.06
OP-B20	14	6	8	42.86%	-	-	380 – 2800	0.136	1.14
OP-C06	12	3	9	25%	-	1	350 – 1750	0.066	1.5
OP-C16	15	8	7	53.33%	-	2	300 - 2950	0.142	0.93
OP-C18	12	4	8	33.33%	-	2	430 - 2330	0.077	1.23
OP-D11	14	5	9	35.71%	-	1	340 – 2390	0.104	1.29
OB-D13	17	12	5	70.58%	-	3	210 – 2230	0.204	0.59
OP-H07	11	3	8	27.27%	-	-	200 – 1380	0.088	1.45
OP-H18	16	7	9	43.75%	2	-	140 – 2100	0.126	1.13
Total Band	**316**	**132**	**184**	**41.77%**	**12**	**19**		**o.119**	**31.18**
Average	**14.36**	**6**	**8.36**	**١.89**	**0.5**	**0.86**	**135-4450**	**o.119**	**1.42**

Polymorphic information content: PIC

Genetic Similarity Analysis Based On RAPD Marker:

The results generated from RAPD profiles were pooled for drawing the relationships among the ten *Hordeum vulgare* L. genotypes under study. The estimated similarities relationships ranged from 0.767 to 0.884. The highest similarity value (0.884) was recorded between both Giza 123 and Giza 126, this indicated that Giza 123 was closely related to Giza 126. On the other hand, the lowest similarity value (0.767) was recorded between Giza 123 and Giza 130, indicating that these were distantly related variety as shown in Figure (4).

Polymorphism as Detected By ISSR Analysis:

These primers generated a total of 159 bands; 98 of these bands were polymorphic (62.02%) (Table 5 and (Figs. 5 & 6). The polymorphic information content (PIC) varied from 0.118 to 0.273 where primers ISSR-11 had the highest PIC values while primer 17898BS5 has the lowest PIC value. ISSR markers characterized 25 unique markers among the ten studied varieties including 8 positive unique ISSR markers and 17 negative unique markers. These unique bands ranged in size from 200 to 3900 bp.

Table 5: Total number of bands, polymorphic bands, percentage of polymorphism, PIC and allele size range as revealed by ISSR analysis.

Primer name	Total number of bands	No. of Polymorphic bands	No. of Monomorphic bands	Polymorphism percentage	No. of Positive unique bands	No. of Negative unique bands	Allele size range (bp.)	PIC	BSI
ISSR-1	15	10	5	66.66%	2	2	480-3150	0.179	0.7
ISSR-3	12	6	6	50%	-	-	230-1330	0.161	1
ISSR-4	13	9	4	69.23%	-	3	350-2050	0.174	0.6
ISSR-8	17	11	6	64.70%	-	1	190-1450	0.194	0.7
ISSR-11	15	12	3	80%	-	1	470-2510	0.273	0.4
ISSR-12	19	12	7	63.16%	2	2	220-3900	0.181	0.7
ISSR-14	17	11	6	64.7%	1	1	140-1650	0.201	0.7
C-2	17	9	8	52.94%	-	4	310-2900	0.129	0.9
17889B	15	10	5	66.66%	2	1	90-1960	0.189	0.7
17898BS5	19	8	11	42.1%	1	2	177-2089	0.118	1.6
Total Band	159	98	61	-	8	**17**	-	1.799	0.8
Average	15.9	9.8	6.1	62.02%	0.8	1.7	90-3900	0.179	0.93

Genetic Similarity Analysis Based On RAPD Marker:

The highest similarity value (0.803) was recorded between Giza 128 and Giza 129 indicating that these two cultivars were closely related to each other. On the other hand, the lowest similarity value (0.618) was recorded between Giza 123 and Giza 129, indicating that these were distantly related cultivars as shown in Figure (7).

.

In the combined similarity matrix of RAPD and ISSR analysis (Fig. 8), the highest similarity value (0.855) was recorded between Giza 123 and Giza 126 this indicating that these two cultivars were closely related to each other. On the other hand, the lowest similarity value (0.729) was recorded between Giza 123 and Giza 129, indicating that these two varieties were the most diverse varieties.

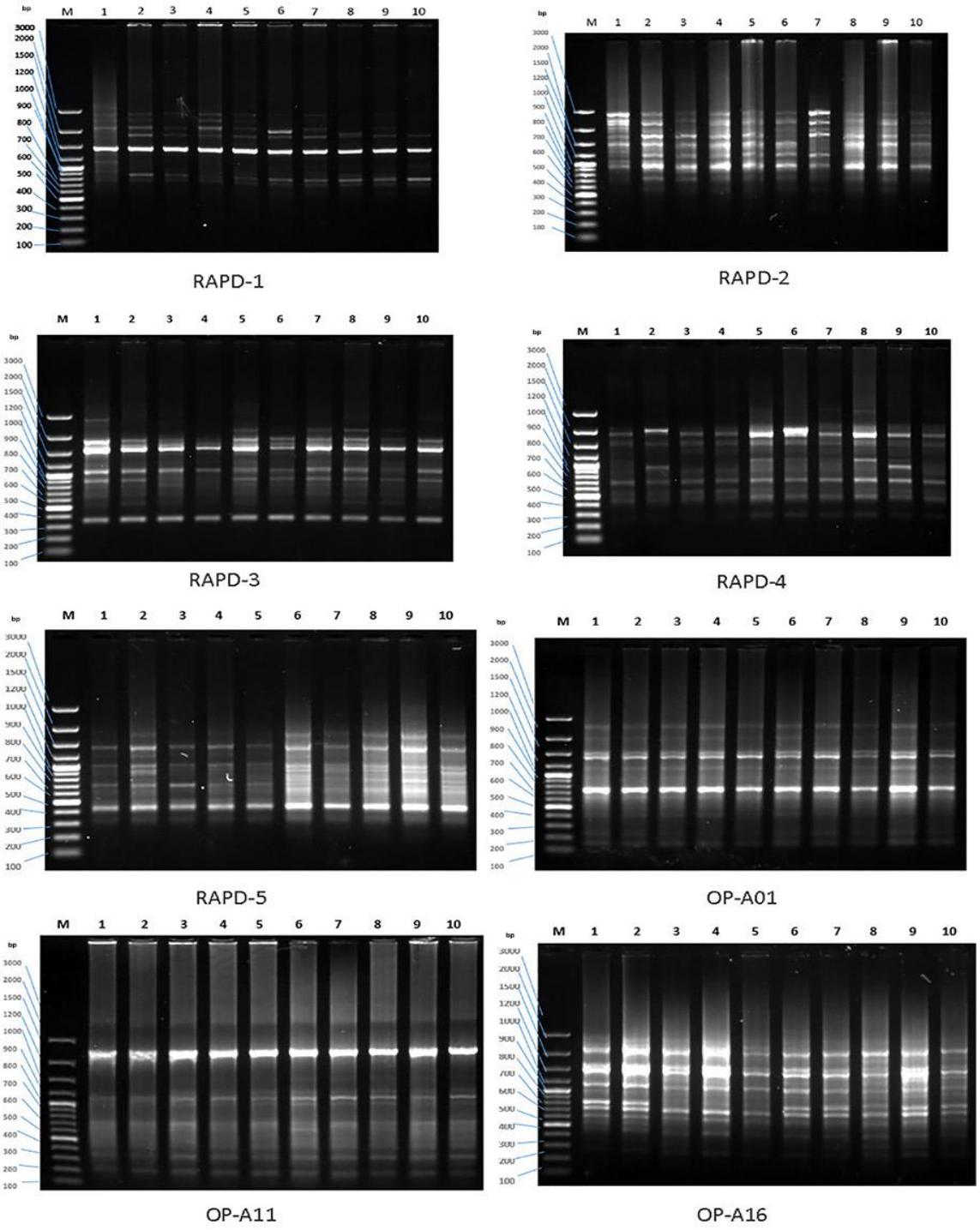

Fig.1: RAPD pattern of ten variety of *Hordeum vulgare* L. (Giza 123, Giza 126, Giza 127, Giza 128, Giza 129, Giza 2000, El-Kheroba, El-Sheikh Zuwaid and Wadi Sedr)

Cultivars: 1-Giza 123, 2-Giza 126, 3-Giza 127, 4-Giza 128, 5-Giza 129, 6-Giza 130 and 7- Giza 2000.
Landraces: 8- El-Kheroba, 9- El-Sheikh Zuwaid and 10- Wadi Sedr.

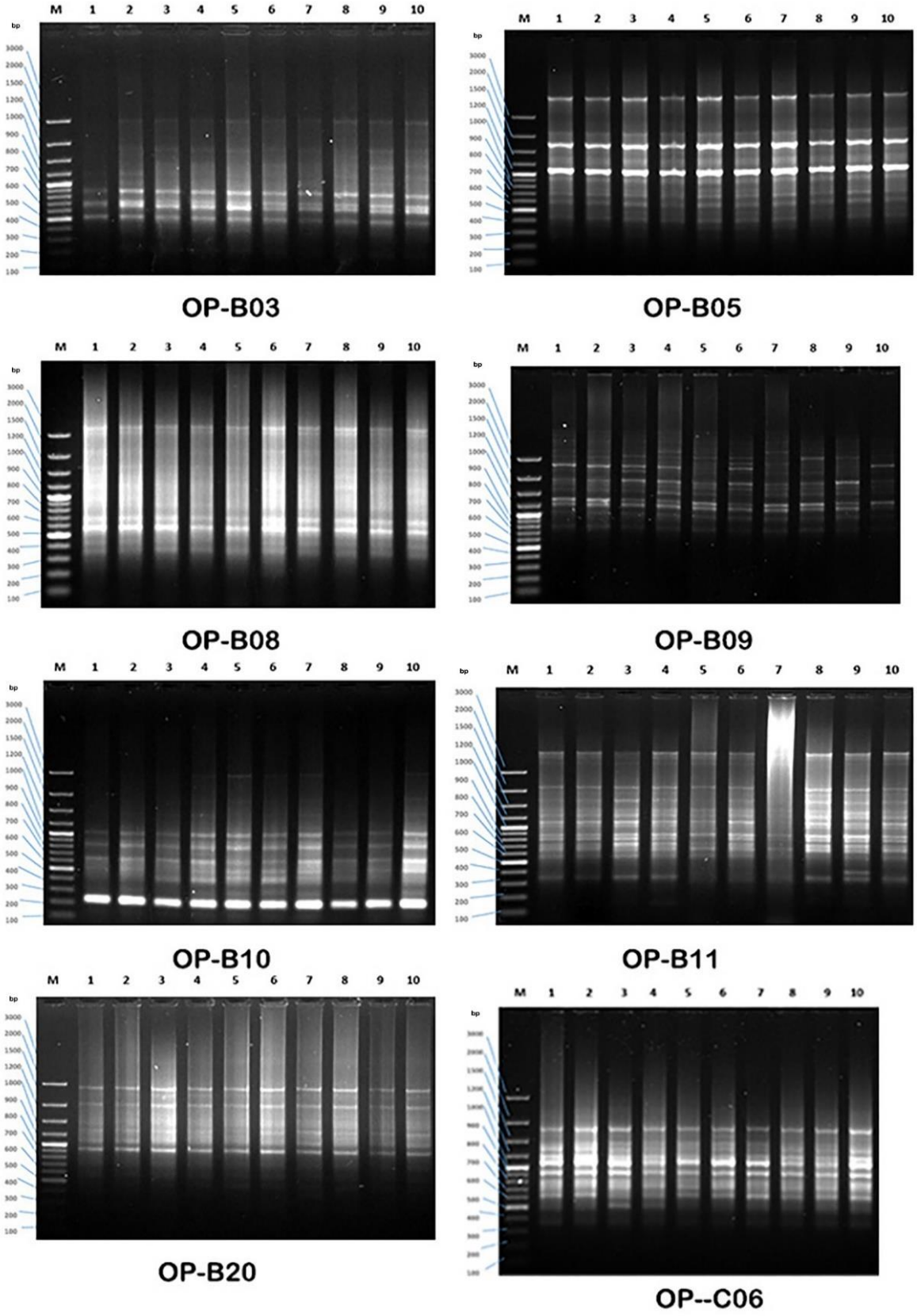

Fig.2: RAPD pattern of ten variety of *Hordeum vulgare* L.

Cultivars: 1-Giza 123, 2-Giza 126, 3-Giza 127, 4-Giza 128, 5-Giza 129, 6-Giza 130 and 7- Giza 2000.
Landraces: 8- El-Kheroba, 9- El-Sheikh Zuwaid and 10- Wadi Sedr.

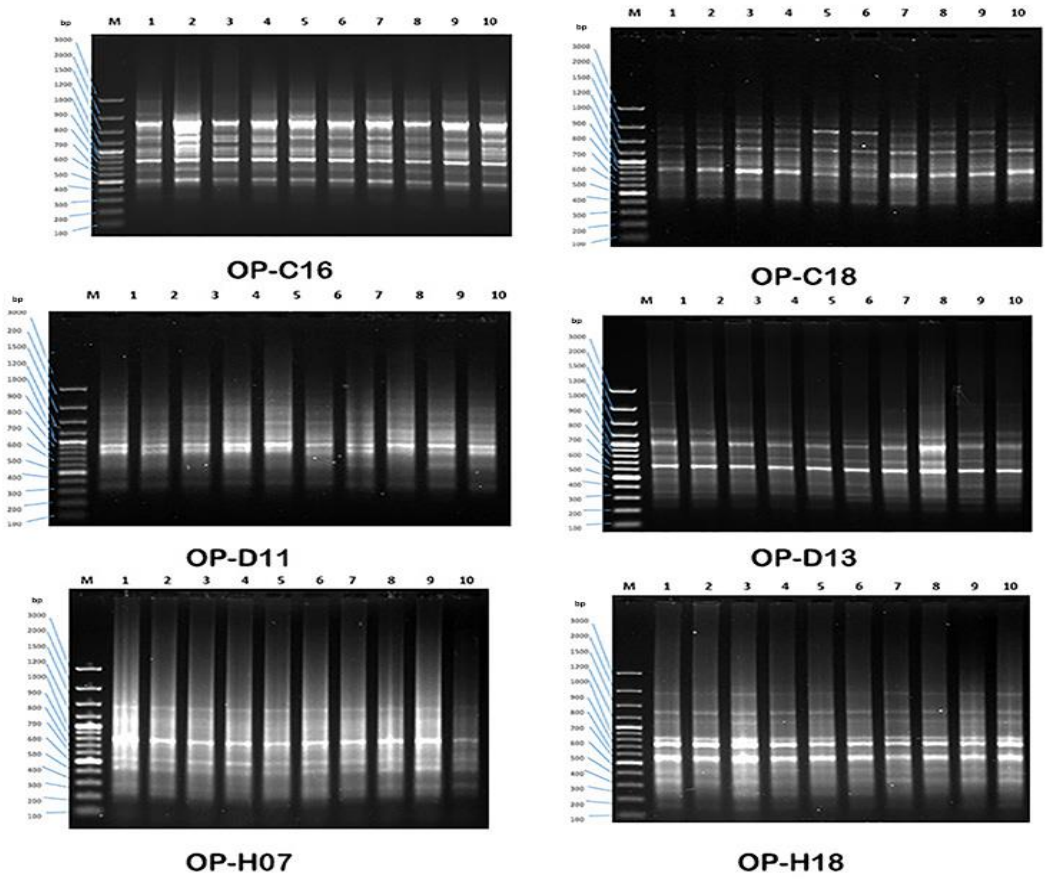

Fig.3: RAPD pattern of ten variety of *Hordeum vulgare* L.

Cultivars: 1-Giza 123, 2-Giza 126, 3-Giza 127, 4-Giza 128, 5-Giza 129, 6-Giza 130 and 7- Giza 2000.
Landraces: 8- El-Kheroba, 9- El-Sheikh Zuwaid and 10- Wadi Sedr.

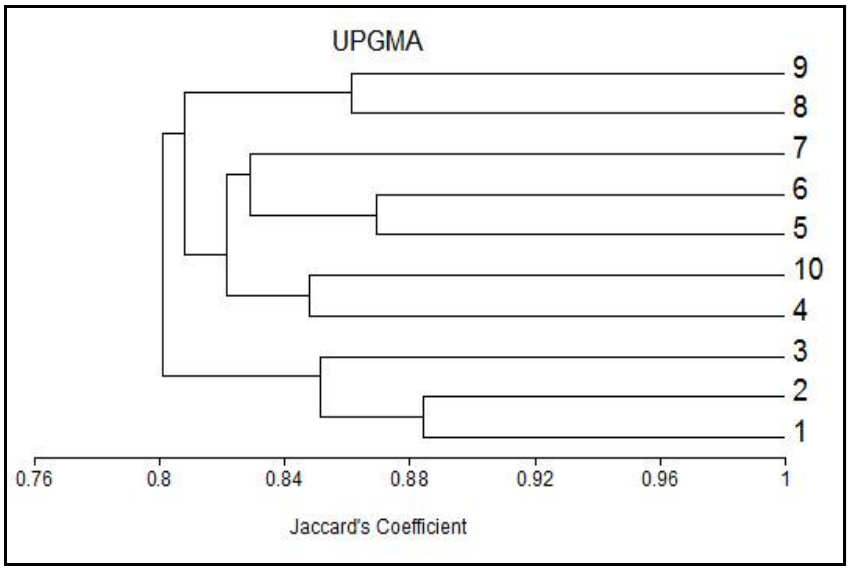

Fig.4:Dendrogram representing the genetic relationship among the ten *Hordeum vulgare* L. varieties using UPGMA cluster analysis of Jacquard's coefficient generated from RAPD markers.

Cultivars: 1-Giza 123, 2-Giza 126, 3-Giza 127, 4-Giza 128, 5-Giza 129, 6-Giza 130, 7- Giza 2000. Landraces varieties: 8- Sinai, El-Aresh, El-Kheroba, 9- Sinai,El-Aresh, El-Sheikh Zuwaid, 10- Sinai, Ras Sedr, Wadi Sedr.

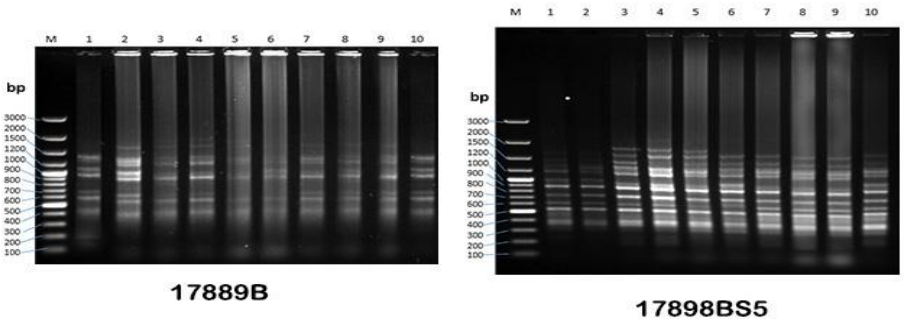

Fig.5: ISSR pattern of ten variety of *Hordeum vulgare* L.

Cultivars: 1-Giza 123, 2-Giza 126, 3-Giza 127, 4-Giza 128, 5-Giza 129, 6-Giza 130 and 7- Giza 2000.
Landraces: 8- El-Kheroba, 9- El-Sheikh Zuwaid and 10- Wadi Sedr.

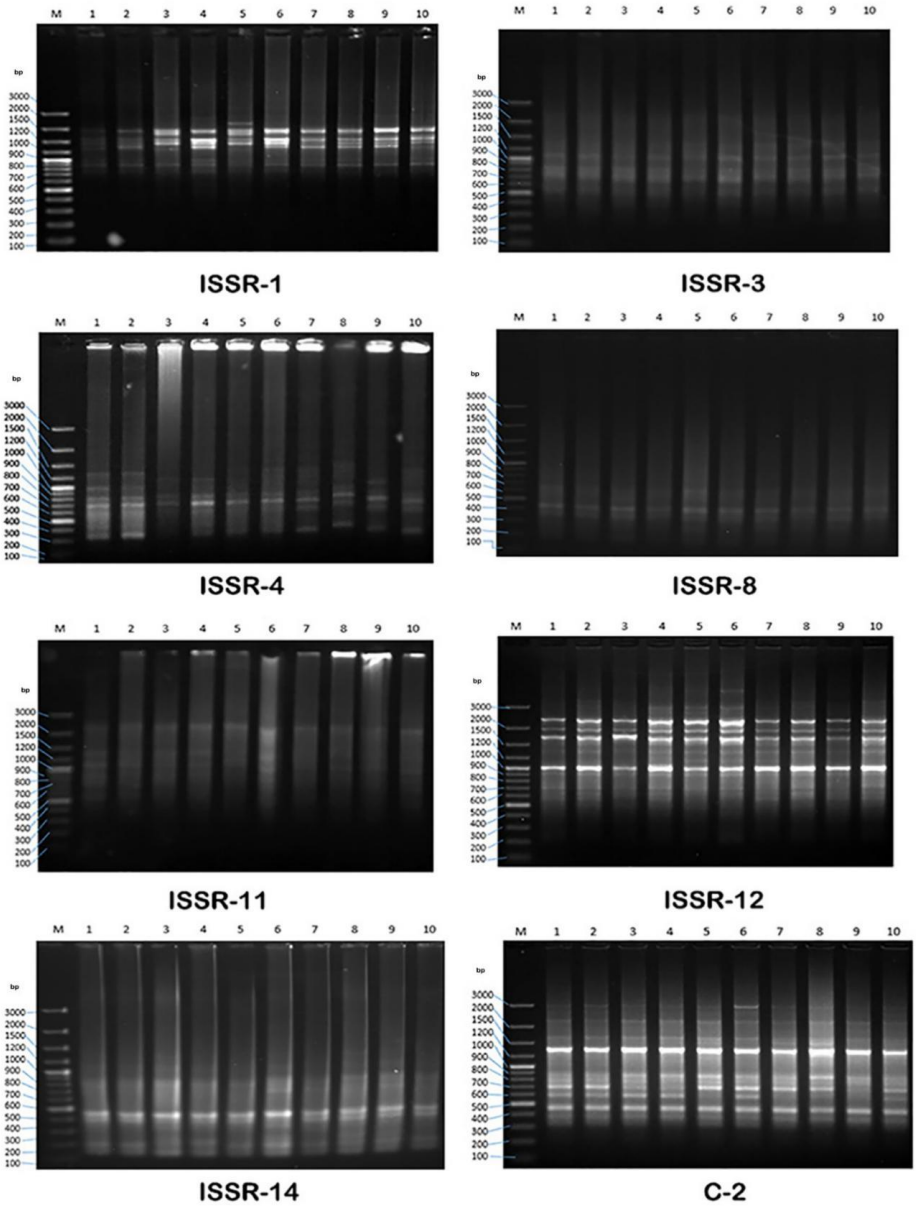

Fig.6: ISSR pattern of ten variety of *Hordeum vulgare* L.

Cultivars: 1-Giza 123, 2-Giza 126, 3-Giza 127, 4-Giza 128, 5-Giza 129, 6-Giza 130 and 7- Giza 2000.
Landraces: 8- El-Kheroba, 9- El-Sheikh Zuwaid and 10- Wadi Sedr.

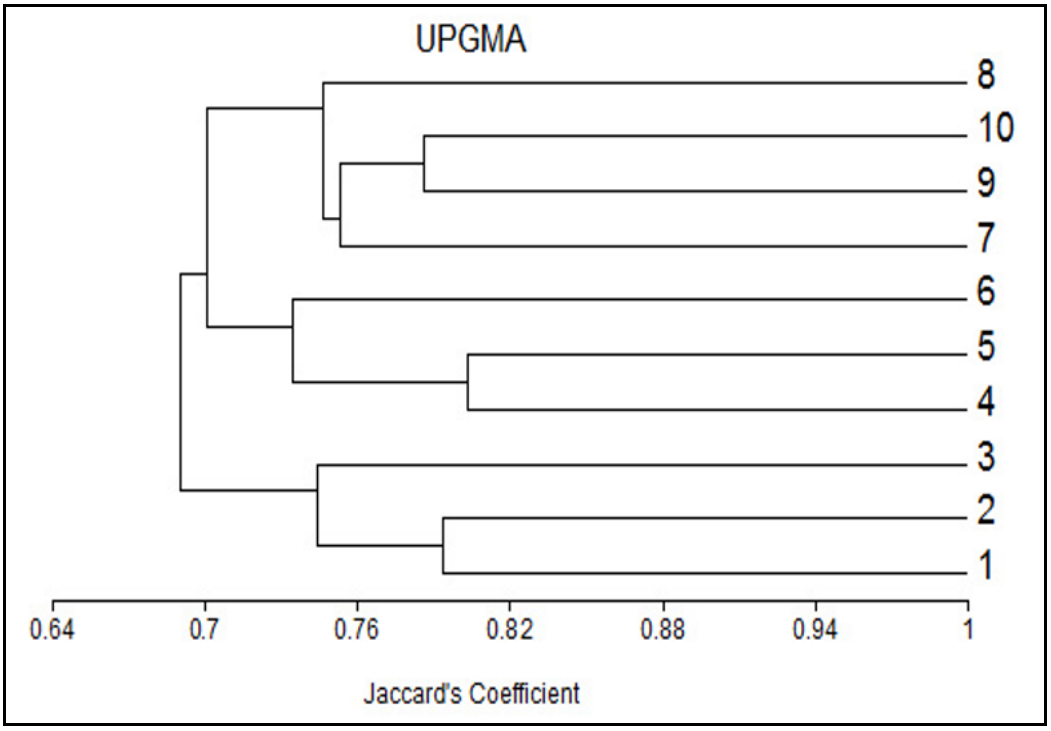

Fig.7: Dendrogram representing the genetic relation-ship among the ten *Hordeum vulgare* L. varieties using UPGMA cluster analysis of Jaccard´s coefficient generated from ISSR markers.

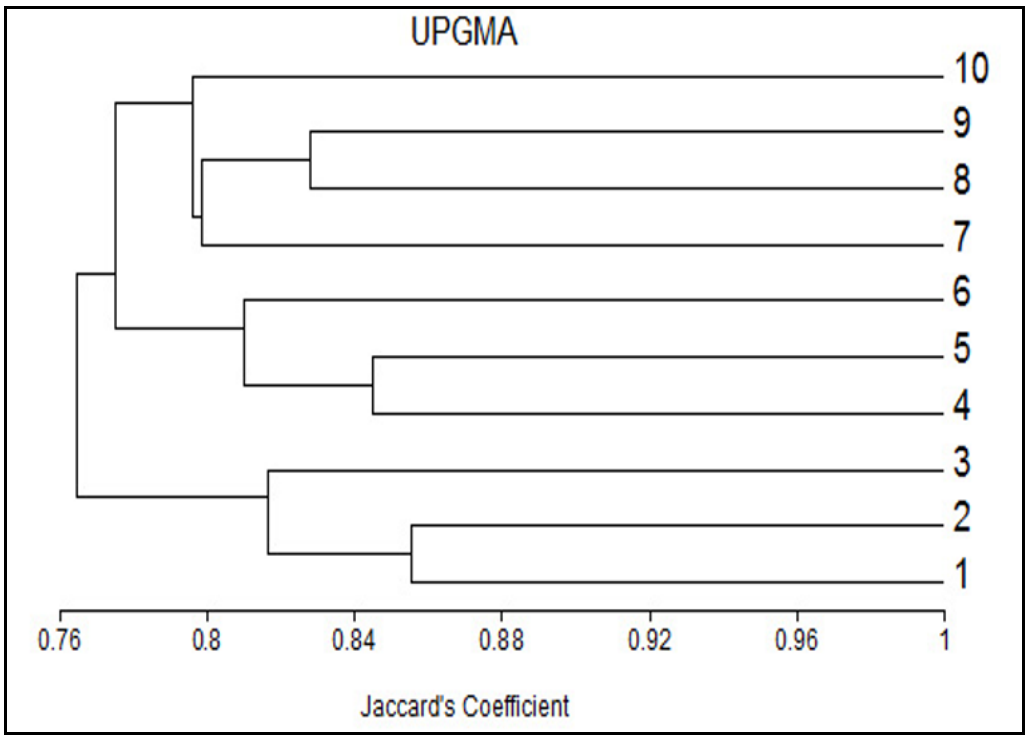

Fig. 8: Dendrogram representing the genetic relationship among the ten *Hordeum vulgare* L. varieties using UPGMA cluster analysis of Jaccard´s coefficient as computed according to RAPD and ISSR markers.

Cultivars: 1-Giza 123, 2-Giza 126, 3-Giza 127, 4-Giza 128, 5-Giza 129, 6-Giza 130, 7- Giza 2000.
Landraces : 8- El-Kheroba, 9- El-Sheikh Zuwaid, 10- Wadi Sedr

DISCUSSION

The techniques, which were used in classification based on molecular markers like ISSR and RAPD provide an accurate and powerful tool in analyzing the genetic relationship. These methods detect polymorphism by assaying subsets of the total amount of DNA banding variation in a genome (Kanbar and Kondo, 2011). Positive and negative unique bands for each marker could be potential for species-specific markers (Roman *et al*., 2003). Heiba *et al*. (2019) found that RAPD, ISSR and SSR markers play a vital and successful role in the identification of different Egyptian wheat and barley genotypes, which considered helpful in the enhancement of cereals production in Egypt.

Two molecular markers were used RAPD and ISSR to analyze different genetic information. ISSR markers are more discriminating than RAPD to evaluate the genetic diversity and relationship among species (Ashraf *et al*., 2013). In spite of RAPD is very quick and easy to develop but its reproducibility is less than ISSR. Kafkas *et al*., 2006 and Jabbar Zadeh, *et al*., 2010 stated that ISSR assays are more reliable than RAPD because of their reproducibility so they may be preferred.

The total percent of polymorphism detected by RAPD and ISSR analysis was 41.77% and 62.02% respectively. These results indicate that ISSR has a high capacity to reveal polymorphism. Also, it has great potential to determine intra and inter-specific variation compared to other arbitrary primers like RAPDs (Zietkiewicz *et al*., 1994 and Agarwal *et al*., 2008).

Nkongolo, *et al*., 2011, stated that ISSR which tends to be found in high copy repeat regions and detects polymorphism in inter-microsatellite loci using primer designed di or tri-nucleotide simple sequence repeats. Also, ISSR is more beneficial than AFLP due to its high specificity and repeatability, in terms of its time-saving quality (Wolfe *et al*., 1998; Blair *et al*., 1999; Fernández *et al.*, 2002 and El-Atroush *et al*., 2014).

RAPD and ISSR have different resolutions and the two marker techniques targeted different regions of the genome (Souframanien and Gopalakrishna, 2004). These differences may also be attributed to the level of polymorphism detected reinforcing the importance of number of loci and their coverage of the whole genome for obtaining reliable estimates of genetic relationships among cultivars. The number of alleles at a locus and their distribution frequency generate polymorphism between species represent the genetic variability (Marzougui, *et al*., 2009).

Variability in band profiles was observed in this study, where some new bands were appeared and others were disappeared. Savva *et al.* (1994) showed that DNA fingerprints will be altered by factors such as exposure of an organism to a genotoxic chemical which may result in the formation of a covalently bound adduct between the chemical and the DNA. Faulty repair of these adducts may prevent the primer from binding to those sites command to mutations and, sometimes to cytogenetic changes.Atienzar and Jha (2004 and 2006) used RAPD technique and confirmed that when the *Taq* DNA polymerase encounters a DNA adduct, there are several possible results including blockage, by-pass and the possible dissociation of the enzyme/adduct the complex which will cause changes in RAPD profiles. Breakages which take place in the DNA template between two opposite primers may result in a loss of an amplicon whereas genetic rearrangements and point mutations may be accountable for either a loss or induction of new annealing sites which could result in the disappearance or the appearance of new amplicons, respectively. The appearance of bands could be referring to the presence of new sites which become accessible to primers after structural modulation in DNA sequence that occurred due to mutations (resulting in new annealing events) or large deletions (bringing two pre-existing annealing sites

closer) or recombination; while the disappearance of bands may be attributed to the presence of DNA adducts, which can act to block or reduce the polymerization of DNA in the PCR-reaction (Jones & Parry, 1992).

Comparison of PIC value for the two marker systems indicated that the range of PIC values for RAPD primers was from 0.204 (OB-D13) to 0.043 (OP-A01), where the range of PIC values for ISSR primers was from 0.273 (ISSR-11) to 0.118 (17898BS5).

It was found that inter simple sequence repeat markers have a high potential to identify polymorphism and determine inter and intragenomic diversity across species as compared to other random primers like RAPD (Souframanien and Gopalakrisha, 2004).

High products of ISSR bands comparing with RAPD could be due to the higher annealing temperatures and longer used primers (Huangfu *et al*., 2009). Also, the climate conditions play a main role in the evaluation of landraces by demonstrating significant levels of variation in response to the selection stress in the regions (Souframanien and Gopalakrishna, 2004).

UPGMA analysis of binary matrices data indicates that there is a very good correlation between ISSR and RAPD based similarities. High similarity indices suggest that the species have a close genetic relationship among them but low similarity indices suggest that the species are distantly related to each other (Hasan *et al*., 2009). The similarity matrices of RAPD and ISSR analysis ranged from 0.78 to o.88 and 0.652 to 0.794 respectively. It may be due to the highly polymorphic, abundant nature of the micro-satellite due to slippage in DNA replication (Tagizad *et al.*, 2010). The uses of two markers in a combination were effective in discriminating the different species.

The UPGMA dendrograms confirmed the difference between ISSR and RAPD; the farmer was able to differentiate well between the ten studied barley cultivars more than the last one. RAPD profile analysis revealed some cultivar-specific bands that could be used to identify the different genotypes of barley cultivars; these include the presence of unique bands in cultivars Giza 123, Giza 126, Giza 129, Giza 130, Giza 2000, El-Kheroba and El-Sheikh Zuwaid and the absence of one band in cultivars Giza 123, Giza 126, Giza 127, Giza 129, Giza 130, Giza 2000, El-Kheroba, El-Sheikh Zuwaid, and Wadi Sedr. These unique bands in the RAPD profile may define as genetic fingerprinting that may be associated with one or more morphological traits. These unique bands may also prove useful for mapping of certain genes that may be associated with some features of the above-mentioned cultivars in future research El-Shazly and El-Mutairi (2006)

The RAPD results in the present studies indicated that a total bands were amplified among these varieties were 316 bands including 132 were polymorphic, Also the number of bands per primer was 14.36 bands this corresponds to a level of polymorphism of 41.77%. These results disagree with the results of Showman *et al.*, (2001), who recorded 3.83 bands per primer using other primers while Yu *et al.*, (2002) reported 9.6 bands per primer in the *Tibetan* hulless barey varieties. Also, Kuczynska *et al.*, (2001) recorded 6.8 bands per primer. Hamidi, (2018) studied the molecular variation of 10 Barley diverse landraces that collected from Iraq using RAPD marker. Her studies indicated that total bands were 81 bands, 51 are polymorphic showing a high level of polymorphism (71.8%).

Changes in bands may be the consequences of genomic template instability (GTS) related to the level of DNA damage, the efficiency of DNA repair and replication (Atienzar *et al*., 2002; Atienzar and Jha, 2004 and Ciğerci *et al*., 2016)

El-Shazly and El-Mutairi (2006) documented from the UPGMA and NJ trees based on RAPD data alone the two-rowed cultivars Local and Giza 128 were separated together from the six-rowed cultivars. This agreement with the present results from UPGMA and

Jacquards' coefficient based on RAPD that two-rowed cultivars Giza 127 and Giza 128 were separated from the other six-rowed studied cultivars, while Hang *et al*., (2000) separated the two-rowed and six-rowed North American barley as two different groups using RAPD fingerprinting

Mostafa *et al*.,2020 found that the genetic dissimilarity tree of studied Egyptian *Hordeum vulgare* L varieties was produced by hierarchical cluster analysis based on morphological data stated the pedigree. However, the genetic similarity tree was produced by UPGMA based on molecular markers (SSRs) and soluble protein patterns were not in full agreement with the pedigree.

According to UPGMA, of the present study, the highest close relationship was recorded between Giza 2000 and (Giza 130 and Giza 129) indicating that Giza 130and Giza 129 were closely related to Giza 2000. On the other hand, Giza 123 cultivar and Wadi Sedr landrace indicating that these were distantly related cultivars as shown in figure (4). Also, unknown landraces Sinai, El-Arish, El-Kheroba and Sinai, El-Arish, El-Sheikh Zuwaid were closely related to Giza 2000, while landrace Sinai, Ras Sedr, Wadi Sedr was closely related to Giza128.

Conclusion:

The present study reveals that ISSR was an accurate marker more than RAPD in the detection of genetic variation among the studied barley varieties. Also, unknown landraces Sinai, El-Arish, El-Kheroba and Sinai, El-Arish, El- genetic distance tree was produced by UPGMA based on molecular markers (RAPD and ISSR) state that, unknown landraces El-Aresh, El-Kheroba and Sinai, El-Arish, El-Sheikh Zuwaid were closely related to Giza 2000, while landrace Sinai, Ras Sedr, Wadi Sedr was closely related to Giza128.

REFERENCES

Agarwal, M.; N. Shrivastava and H. Padha (2008). Advances in molecular marker techniques and their applications in plant sciences. Plant Cell Report, 27 (4): 617-631.

Anderson, J. A.; G. A. Churchil; J. E. Autrique; S. O. Tanksley and M. E. Sorrels (1993). Optimizing parent selection for genetic linkage maps. Genome, 36: 181-186.

Ashraf, M.; N. A. Akram; M. Ur-Rahman and M. R. Foolad (2013). Marker assisted selection in plant breeding for salinity tolerance. Methods in Mol. Biol. , 913: 305-333.

Atienzar, F.A. and Jha, A.N. (2006). The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: a critical review. Mutat. Res.,613, 76-102.

Atienzar, F.A. and Jha, A.N. (2004) The random amplified polymorphic DNA (RAPD) assay to determine DNa alterations, repair and transgenerational effects in B(a)P exposed *Daphnia magna*. Mutat. Res., 552, 125-140..

Atienzar, F.A., Venier, P. and Jha, A.N. (2002).Evaluation of the random amplified polymorphic DNA (RAPD) assay for the detection of DNa damage and mutations. Mutat. Res. eri populations detected by inter-simple sequence repeats. Genet. Mol. Res., 14(1), 619-625

Bardakci, F. (2001). Random Amplified Polymorphic DNA (RAPD) Markers. *Turk J Biol.,* 25: 185-196.

Blair, M. W.; O. Panaud and S. R. McCouch (1999). Inter simple sequence repeat (ISSR) amplification for analysis of microsatiellite motif frequence and fingerprinting in rice (*Oryza sativa* L.). *Theor. and Appl. Genet. , 98 (5): 780-792.*

Brantestam, A. K.; R. von Bothmer; C. Dayteg; I. Rashal; S. Tuvesson and J. Weibull (2004). Inter simple sequence repeat analysis of genetic diversity and relationships in cultivated barley of Nordic and Baltic origin. Hereditas, 141 (2): 186–192.

Ciğerci, I.H., Cenkci, S., Kargioglu, M. and Konuk, M. (2016) Genotoxicity of *Thermopsis Turcica*on *Allium cepa*.

roots by alkaline comet and random amplified polymorphicDNA assays. Cytotech., 68, 829-838.

El-Atroush, H.; H. M Barakat; M. A. Tantawy and A. E. El-Shabasy (2014). Molecular Characterization of Nitraria retusa and selected taxa of Zygophyllaceae using RAPD and ISSR markers. Egypt. of Genet. and Cytol. (accept. 1/10/2014).

El-Atroush. H.; A. E. El-Shabasy; M. A. Tantawy and H. M. S. Barakat (2015). Pollen morphology and protein pattern of *Nitraria retusa* and some selected taxa of Zygophyllaceae in Egypt. Egypt. J. Bot., 55 (2): 207-230.

El-Shazly, H. H. and Z. El-Mutairi (2006). Genetic relatinships of some barley cultivars, based on morphological criteria and RAPD fingerprinting. Intern. J. of Bot., 2 (3): 252-260.

Fernández, M. E.; A. M. Figueiras and C. Benito (2002). The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin. Theor. and Appl. Genet., 104 (5): 845-851.

Goyat, S.; A. Grewal; D. Singh; R. S. Katiyar; S. K. Tewari; R. C. Nainwal and K. H. Bindu (2016). Evaluation of genetic diversity of *Piper betle* cultivars using ISSR markers. Inter.l J. of adv. Res. (IJAR), 4 (1): 571-579.

Hamidi, H. (2018). Genetic diversity study in some Barley diverse landraces based on RAPD markers in Kurdistan region of Iraq. Intl J Farm & Alli Sci., 7 (4): 100-106

Hang, A.; C. S. Burton and D. L. Hoffman (2000). Random amplified polymorphic primer generated embryo DNA polymorphisms among 16 North American malting barley cultivars. J. of Brew. Chem., 58: 147-151.

Harlan, J. R. and D. Zohary (1966). Distribution of wild wheats and barley. Sci., 153 (3740): 1074- 1080.

Hasan, S. M. Z.; M. S. B. Shafie and R. M. Shah (2009). Analysis of random amplified polymorphic DNA (RAPD) of *Artemisia capillaries* (Worm wood capillary) in east coast of Penisular Malaysia. World Appl. Sci. J., 6 (7): 976-986.

Heiba, S.A.A.; A.A.A. Haiba and H.M. Abdel-Rahman (2019). Determination of Genetic Markers in Some Egyptian Varieties of wheat and Barley under Salt and Drought Stresses. Asian J. of Crop Sci., 11(2): 59-70.

Huangfu, C-H.; X-L. Song and S. Qiang (2009). ISSR variation within and among wild *Brassica juncea* population implication for herbicide resistance evolution. Genetic Reso. and Crop Evol. , 56 (7): 913-924.

Jabbarzadeh, Z.; M. Khosh-khui; H. Salehi and A. Saberivand (2010). Inter simple sequence repeat (ISSR) markers as reproducible and specific tools for genetic diversity analysis of rose species. Afri. J. Biotech, 9 (37): 6091-6095.

Jones, N.J. and Parry, J.M. (1992)The detection of DNA adducts, DNA base changes and chromosome damage for the assessment of exposure to genotoxic pollutants. Aquat. Toxicol.,22: 323-344.

Kafkas, S.; H. Ozkan; I. Acar; H. S. Atli and S. Koyuncu (2006). Detecting DNA polymorphism and genetic diversity in a wide *Pistachio* germplasm: comparison of AFLP, ISSR, RAPD markers. J. of the Amer. Soc. for Horti. Sci., 131 (4): 522 – 529.

Kanbar, A. and K. Kondo (2011). Efficiency of ISSR and RAPD dominant markers in assessing genetic diversity among Japanese and Syrian cultivars of barley (*H. Vulgare* L.). Res. J. of Agri. and Biol Sci, 7 (1): 4-10.

Kuczynska, A.; P. Milczarski; M. Surma; P Masojc and T. Adamski (2001). Genetic diversity among cultivars of spring barley revealed by random amplified polymorphic DNA (RAPD). J. of Appl. Genet., 42 (1): 43-48.

Liu, K. and S. V. Muse (2005). Power

Marker: Integrated analysis environment for genetic marker data. Bioinform., 21: 2128-2129.

Martin, J.P. and M.D. Sanchez-Yelamo (2000). Genetic relationships among species of the genus Diplotaxis (Brassicaceae) using intersimple sequence repeat markers. Theor Appl Genet., 101: 1234-1241

Marzougui, N.; A. Boubaya; W. Elfalleh; F. Guasmi; L. Laaraiedh; A. Ferchichi; T. Triki and M. Beji (2009). Assessment of genetic diversity in *Tigonella foenum graecum* Tunisian cultivars using ISSR markers. J.of Agri.ad Env, 7 (1): 101-105.

Moreno, S.; J.P. Martin and J.M. Ortiz (1998). Inter-simple sequence repeats PCR for characterization of closely related grapevine germplasm. Euphytica, 101: 117–125.

Mostafa, E.A.H., El-Ashry. Z.M. , F. I. Mohamed F.I.,S. E. El-Khodary,S.E. Osman, S.A. and El-Atroush, H.(2020): Phenetic and Phylogenetic Relationships of Egyptian *Hordeum vulgare* L. Using Agro-morphological Criteria, Molecular Marker and Genes Expression. Takhol. 40:27-40

Mylonas, G. I.; A. P. Apostolidis; M. Koutsika-Sotiriou; A. Georgiadis and K. Bladenopoulos (2014). Barley cultivar discrimination and hybrid purity control using RAPD markers. Rom. Biotechnol. Letter., 19 (3): 9421-9428.

Nguyen, T. T.; P. W. J. Taylor; R. J. Redden and R. Ford (2004). Genetic diversity estimates in *Cicer* using AFLP analysis. Plant Breed., 123 (2): 173–179.

Nkongolo, K. K.; K. Mbuya; M. M. Smith and A. Khlonji-Mbuyi (2011). Molecular analysis of quality protein (QPM) and normal maize varieyies from DR-Congo breeding program. Afri. J. of Biotech., 10 (65): 14293-14301.

Roman, B.; C. Alfaro; A. M. Torres; M. T. Moreno; Z. Satovic; A. Pujadas and D. Rubiales (2003). Genetic relationships among *Orobanche* species as revealed by RAPD analysis. Annals Bot., 91 (6): 637-642.

Salimath, S.S.; A.C. De Oliveira; I.D. Godwin and J.L. Bennetzen (1995). Assessment of genome origins and genetic diversity in the genus Eleusine with DNA markers. Genome, 38: 757–763.

Savva D. (2000**)** The use of arbitrarily primed PCR (AP-PCR) fingerprinting detects exposure to genotoxic chemicals. Ecotoxicol., 9: 341 – 353.

Savva, D., Castellani, S., Mattei, N., Renzoni, A. and Walker, C.H. (1994). The use of PCR and DNA fingerprints to detect the genotoxic effects of environmental chemicals. In: Environmental Contamination, S.P. Varnavas (ed.), (CEP Consultants, Edinburgh), 105-110.

Sharma, P.; S. Tiwari; N. Tripathi and A. K. Mehta (2016). Polymorphism analysis in advanced mutant population of oat (*Avena sativa* L.) using ISSR markers. Physiology and Mol. Biol.of Plants, 22 (1): 115-120.

Showman, W.; S. A. Ghazal and M. Baum (2001). Genetic diversity in Syrian barley using RAPD markers. College of Agriculture, King Saud Univ. Res. Brochure. 99: 50-290.

Souframanien, J. and T. Gopalakrishna (2004). A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. Theor and appl. Genet., 109 (8): 1687-1693.

Tagizad, A.; J. Ahmadi; R. Haddad and M. Zarrabi (2010). A comparative analysis of ISSR and RAPD markers for studying genetic diversity in Iranian *Pistachio* cultivars. Iran. J. of Genetics and Plant Breed., 1 (1): 6-16.

Wang A.; Z.Yu and Y.Ding (2009). Genetic diversity analysis of wild close relatives of barley from Tibet and the Middle East by ISSR and SSR markers. C. R. Biologies, 332: 393–

403.

Williams, J.G.K.; A.R. Kubelik; K.L. Livak; J.A. Rafalski and S.V. Tingey (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531–6553.

Wolfe, A. D.; Q-Y. Xiang and S. R. Kephart (1998). Assessing hybridization in natural populations of Penstemon (Scrophulariacea) using hypervariable intersimple sequence repeat (ISSR) bands. Mole. Ecol., 7 (9): 1107-1125.

Yu, Z.; L. Li-Qiong; L. Huan; B. Jie; Y. Man-Ye; M. Chen; C. Ying-Fan; Q. Xiao-Lin and C. Fang (2002). RAPD markers in diversity detection and variety identification of Tibetan hulless barley. Plant Mol. Biol. Reporter, 20 (4):369-377.

Zietkiewicz, E.; A. Rafalski and D. Labuda (1994). Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. Genomics, 20 (2): 176-183.