Studying sunflower (*Helianthus annuus* L.) genetic diversity using RAPD and ISSR techniques

Dr. Lobna Mokrani*

Abstract

RAPD and ISSR techniques were employed in this research to study the genetic diversity within twenty-nine local and introduced sunflower (*Helianthus annuus* L.) genotypes. Thirteen primers were used for RAPD, which generated 74 molecular markers, 60 of them were polymorphic. On the other hand, the 24 primer pairs used for ISSR generated 129 molecular markers with only25 polymorphic markers. Percent Disagreement Values (PDVs) between genotypes ranged between 0.01 and 0.27 for RAPD and between 0.01 and 0.07 for ISSR. Combined data revealed PDVs ranging between 0.02 and 0.13. Polymorphic Information Content (PIC) values reached 0.31, with an average of 0.13 and 0.03 for RAPD and ISSR respectively. These results

* Lecturer at the department of plant biology – Faculty of sciences – Damascus University.

show that ISSR is not suitable for sunflower fingerprinting since it provides low degrees of polymorphism, which makes RAPD more appropriate. Finally, combining different types of markers is necessaryto overcome the problem of monomorphism and for maximum coverage of a genome.

Key words: Sunflower (Helianthus annuusL.), RAPD, ISSR, Genetic diversity.

دراسة التنوّع الوراثي لدى نبات دوّار الشمس (*Helianthus annuus* L.) باستعمال تقانتيRAPD و ISSR

د. لبنى مقراني*

الملخص

استعملت تقانتا RAPD و ISSR في هذا البحث من أجل دراسة التنوّع الوراثي ضمن 29نمطًا وراثيًا محليًّا ومُدخلا من دوّار الشمس(*Helianthus annuus*L.). ولقد اعتمدت تقانة RAPD على 13 مرئسة أعطت 74 مؤشرًا جزيئيًا، من بينها 60 مؤشرًا متابينًا. كما تمّ استعمال 24 شفعا من مرئسات ISSRالتي أنتجت 129 مؤشرا جزيئيًا لم يكن بينها سوى 25 مؤشرا متباينا. تراوحت قيم نسب عدم التوافق PDV بين 0.01 و 0.27 بالنسبة لتقانة RAPD وبين 0.01 و 0.07 في تقانة ISSR. أمّا تحليل مجموع المؤشرات الناتجة عن التقانتين فقد أعطى نسب عدم توافق ما بين 0.02 و 0.13. من ناحية أخرى أظهرت نتائج التباين الداخلي PIC قيما وصلت إلى 0.31 مع متوسط قدره 0.13 لدى تقانة RAPD، في حين كانت أعلى قيمة لـ PIC 0.14 فقط في تقانة ISSR، كما كان متوسط PIC منخفضا جدا (0.03). تظهر هذه النتائج أنّ تقانة ISSR غير مناسبة لدراسة البصمة الوراثية لدى نبات دوّار الشمس بسبب نسب التباين المنخفضة التي تعطيها، مما يجعل تقانة RAPD أكثر ملاءمة لذلك. ويعد دمج أنواع مختلفة من المؤشرات الجزيئية ضروريا لتجاوز مشكلة التماثل الشكلي للمؤشرات ولتغطية أكبر جزء من المادة الوراثية.

الكلمات المفتاحية: دوّار الشمس (*Helianthus annuus*L.)، RAPD، ISSR، تنوّع وراثي.

* مدرّسة في قسم علم الحياة النباتية – كلية العلوم – جامعة دمشق.

Introduction:

Sunflower (*Helianthus annuus* L.) is one of the most important sources of edible oil in the world. The percentage of sunflower seeds oil ranges between 40% and 50% [1]. Therefore, the main objective of sunflower breeding programs isthe development of productive cultivars with high oil yield and good oil quality. However, studying the genetic diversity is an indispensable preliminary step to evaluate the genetic material before starting a breeding program. Molecular markers proved to be valuable tools in the characterization of genetic diversity between sunflower genotypes [2, 3, 4, 5]. They were also employed in sunflower breeding programs, like marker assisted selection (MAS), using genetic map [6, 7, 8] and Quantitative Trait Loci (QTL) analyses results [9, 10, 11].

However, molecular markers have many technical differences in terms of cost, speed, amount of DNA needed, technical labor, degree of polymorphism, precision of genetic distance estimates and the statistical power of tests [12]. Comparisons between techniques have already been realized for many crops [12, 13, 14]. The preliminary evaluation of techniques would save time and effort, because it allows a best choice of methods in the future. Random Amplified Polymorphic DNA (RAPD), which provides dominant markers,has been used inmany analyses in several crops [15, 16, 17]. It is suitable for DNA fingerprinting despite its lack of reproducibility due to mismatch annealing [18]. Furthermore,

Inter Simple Sequence Repeats (ISSR), recognized as a simple and quick method, was also employed in many genetic diversity studies [13,14].

Genetic diversity could be estimated by different statistical tests. It could also be presented by different ways, such as Percent Disagreement Values (PDVs), distance matrix, tree clustering diagram and neighbor joining stars or groups. Furthermore, Polymorphic Information Content (PIC), Effective Multiplex Ratio (EMR) and Marker Index (MI) are other statistical tools employed to evaluate the polymorphism obtained by different techniques or primers [12, 13, 19, 20, 21]. It is important to mention that contrary to PIC, which expresses the degree of polymorphism between genotypes, EMR expresses the degree of polymorphism between markers (molecular weight levels) obtained by the same primer. Thus, PIC corresponds to the vertical polymorphism and EMR to the horizontal polymorphism. The total polymorphism corresponding to the product of PIC and EMR is called Marker Index (MI). Generally, techniques providing high degrees of total polymorphism (high MI) are the best to be employed whatever the objective of the research.

It is well known that polymorphism depends mostly on the techniques of molecular analyses. Therefore, low degrees of polymorphism could be the result of a bad choice of the technique (e.g. employing SSRs with

genetic materials that contain limited units of microsatellites) and not due to real genetic similarity. Incorrect results could then be obtained. Consequently, it is indispensable to estimate the capacity of each technique to reveal polymorphism before employing it [21]. In conclusion, using more than one technique [13] would ameliorate the polymorphism estimation.

In this study, two techniques, RAPD and ISSR providing dominant markers, were compared in terms of their capacity to reveal polymorphism and to determine the genetic diversity among some sunflower genotypes.

1- Materials and methods

Plant material

Twenty nine sunflower genotypes were used in this research. They include Syrian and introduced varieties (GCSAR) (Table. 1). Seeds were sown in pots in the greenhouse and leaf tissues were obtained from 8 day-old plants. Three reference plants were used:*Calendula officinalis, Tagets sp., Cosmos* sp.

DNA extraction

Total DNA was extracted from sunflower leaves using the micro CTAB procedure [22]. A Gene Quant (Pharmacia Biotech) spectrophotometer was used for DNA quantification and a 0.7% agarose gel for DNA quality detection.

Table1 Sunflower(*Helianthus annuus* L.) genotypes used in the study.

Lane	Genotype code	Genotype	Source
1	A	BaladiHalab	Local market, Aleppo
2	B	Brazili	Local market, Aleppo
3	**C**	Sourgaya4	(GCSAR)* / Local variety
4	D	TarkibiQunaitra	(GCSAR)* / Local variety
5	E	Hysum33	(GCSAR)* / Australian hybrid
6	**F**	434	(GCSAR)* / Greek hybrid
7	G	436	(GCSAR)* / Greek hybrid
8	H	440	(GCSAR)* / Greek hybrid
9	**I**	441	(GCSAR)* / Greek hybrid
10	J	443	(GCSAR)* / Greek hybrid
11	K	KASOL	(GCSAR)* / Italian
12	**L**	7182	(GCSAR)* / Greek hybrid
13	M	7183	(GCSAR)* / Greek hybrid
14	N	7184	(GCSAR)* / Greek hybrid
15	**O**	7185	(GCSAR)* / Greek hybrid
16	P	7186	(GCSAR)* / Greek hybrid
17	**Q**	7187	(GCSAR)* / Greek hybrid

18	**R**	7189	(GCSAR)* / Greek hybrid
19	S	7190	(GCSAR)* / Greek hybrid
20	T	7191	(GCSAR)* / Greek hybrid
21	**U**	7192	(GCSAR)* / Greek hybrid
22	V	BaladiQurdouba	Local market
23	W1	Ghab1	Local market, Ghab
24	W2	Ghab2	Local market
25	W3	Ghab3	Local market
26	W4	Ghab4	Local market
27	W5	Ghab5	Local market
28	X	SfiraHalab	Local market, Aleppo
29	Y	MadkhHalab	Local market, Aleppo
30	Reference plant	*Calendula officinalis*	Doubaya, Yaafour
31	Reference plant	*Tagetssp*	Doubaya, Yaafour
32	Reference plant	*Cosmos sp*	Doubaya, Yaafour

* (GCSAR) General Commission for Scientific Agricultural Research, Douma, Syria.

Molecular analysis

RAPD

Thirteen primers (Operon Technologies Inc. USA and Amersham) were used(Table 2). Amplification reactions were carried out in 12.5 µl volumes containing 50 mM $(NH4)_2SO_4$ (pH 8.8 at 25 C°), 100 mMTris-HCl (pH 8.4 at 25°C), 3.2mM $MgSO_4$, 0.00002% Tween20, 0.005% gelatin (Fluka), 0.25 mM of each dNTP (Roche), 1 unit of *Taq*

polymerase (Fermentas), 42 ng of genomic DNA and 60 ngof each primer. Using a Genius Hybaid Thermal Cycler (Techne, UK), these reactions were subjected to a cycle of 1 min at 94°C followed by 45 cycles, each of which consisted of 10 s at 94°C, 10 s at 35 °C, and 70 s at 72°C. The last cycle was followed by an incubation period at 72°C for 2 min. Amplification products were stored at 4°C until visualization on gel electrophoresis in 1.2% agarose (Q-BIOgene) (to which ethidium bromide (Fluka) was added) using 0.5X Tris Borate EDTA (TBE) buffer. Electrophoresis was performed at 85 V for 2h. A 100 bp ladder (Vivantis) was used to estimate the approximate molecular weight of amplification products.

ISSR

Using 24 selected primers (Table 3), ISSR analysis [23] was carried out on our samples. The amplification was carried out in a 25 µl reaction volume containing 100 mMTris-HCl (pH 8.3), 50 mM $(NH_4)_2SO_4$ (pH 8.8 at 25°C) , 3.2 mM $Mg(SO_4)$, 0.00002% Tween20, 0.25 mM of each dNTP (Roche), 1 unit of Taq DNA polymerase (Fermentas), 28 ng of genomic DNA, 150 Pmol from each primer (Invitrogen and Alfa DNA). Samples were subjected to an initial denaturation at 94°C for 2 min, followed by 40 cycles of 94°C for 10 s, 50°C for 10 s, and 72°C for 10 s. A final extension at 72°C was carried out for 10 min; Genius Hybaid Thermal Cycler (Techne, UK) was used.

Amplification products were size separated by standard horizontal electrophoresis on a 1.8 % agarose (Q-BIOgene) (to which Ethidium bromide (Fluka) was added) in a 0.5 X (TBE) buffer. Electrophoresis was performed at 85 V for 2.30 h. A 100bp DNA ladder (Vivantis) was used to estimate the approximate molecular weight of DNA bands for each amplification product.

Amplification profiles generated by each technique were screened and photographed under UV light. Since more than one replicate was prepared for each primer, only bright and reproducible bands were scored as present (1) or absent (0).

Data analyses:

Matrices of PDV and the resultant cluster analyses were performed using unweighted pair group method with arithmetic averages (UPGMA) of STATISTICA program [24].

Calculations of PIC, appropriate to dominant markers, were done using the formula previously proposed by Rolda N-Ruiz *et al*. [25] :PICi = 2fi (1-fi), where:

PICi is the polymorphic information content for marker i.

fi the frequency of the marker bands which were present.

(1-fi) the frequency of marker bands which were absent.

Dominant markers have a maximum PIC of 0.5 when half of the genotypes have the band and the other half does not have the band. PIC

was averaged over the bands for each primer. MI was calculated as proposed by Powell *et al*. [26]and used byMilbourne*et al*. [27]where MI is the product of diversity index and EMR, where EMR,in turn, is defined as the product of the fraction of polymorphic loci and the number of polymorphic loci. This parameter was calculated for each primer.

2-Results:

Molecular analysis:

The thirteen primers used for RAPD generated 74 markers, 60 of which were polymorphic, representing 81.1% of the total number of markers (a representative gel is shown in Figure 1). The highest number of bands (11) was obtained with R04 primer, however the lowest one (2) with Z14. Single bands were specific to certain genotypes (Brazili, Baladi halab, 436 and 434) (Table 2). On the other hand, the 24 primer pairs used for ISSR technique generated 129 markers with only 25 markers being polymorphic(a representative gel is shown in Figure 2). They represent only 19.4 % of the total number of markers. The highest number of bands (8) was obtained with A4 and C26 primers, however the lowest one (3) with A26 and 164/1. Five single bands characterized some genotypes (Baladi halab with two primers, Ghab 5, Hysum 33 and 7182) (Table 3).

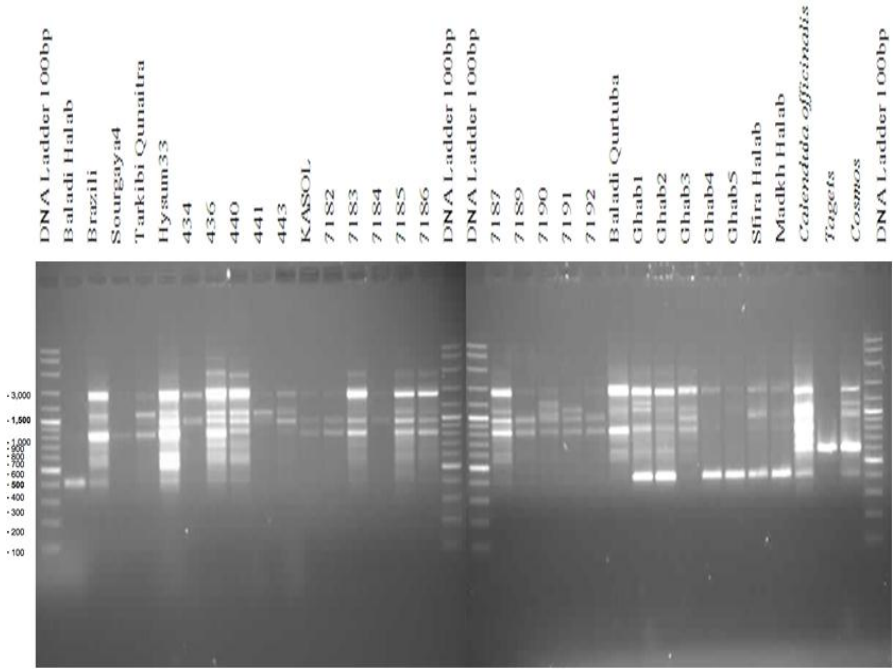


Fig. 1 An agarose gel electrophoresis showing polymorphism resultant from the use of RAPD primer (OP-R04) on 29genotypes and 3positive controls.

Table 2 -Names and RAPD primer sequences and number of polymorphic lines.

Primers	Sequence (5′-3′)	No. Of Band lines	No. Of Polymorphic lines	% Polymorphic lines	Genotype with single bands	PIC	EMR	MI
OP-D08	GTGTGCCCCA	4	4	100	-	0.16	3.57	0.59
OP-E01	CCCAAGGTCC	4	3	75	Brazili (1200-1500 bp)	0.03	1.00	0.03
OP-E12	TTATCGCCCC	7	7	100	-	0.25	1.56	0.40
OP-E18	GGACTGCAGA	6	5	83	Baldi halab (1200-1500 bp)	0.17	3.57	0.59
OP-R04	CCCGTAGCAC	11	11	100	436 (2500-3000 bp)	0.31	11.00	3.36
OP-R07	ACTGGCCTGA	6	5	83	434 (600bp)	0.08	3.13	0.25
OP-R08	CCCGTTGCCT	5	3	60	-	0.04	1.13	0.04
OP-R11	GTAGCCGTCT	7	7	100	-	0.15	6.13	0.94
OP-R15	GGACAACGAG	7	3	43	-	0.09	1.13	0.11
OP-Z 03	CAGCACCGCA	4	3	75	-	0.03	1.50	0.05
OP-Z 13	GACTAAGCCC	5	4	80	-	0.20	3.20	0.64
OP-Z 14	TCGGAGGTTC	2	0	0	-	0.00	0.00	0.00
OP-Z 19	GTGCGAGCAA	6	5	83	-	0.14	2.78	0.38
Total		74	60	81.1				

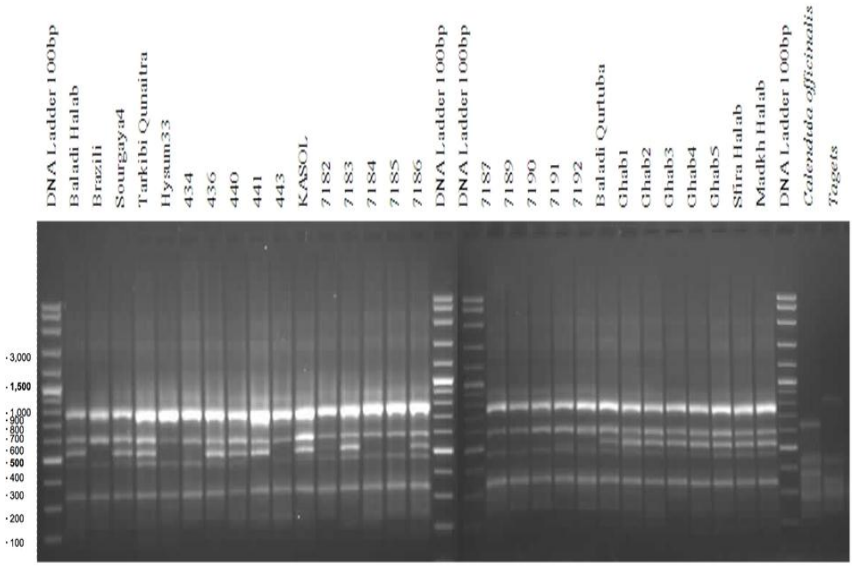


Fig. 2Anagarose gel electrophoresis showingpolymorphism resultant from the use of ISSR primer (B1) on 29genotypes and 2 positive controls.

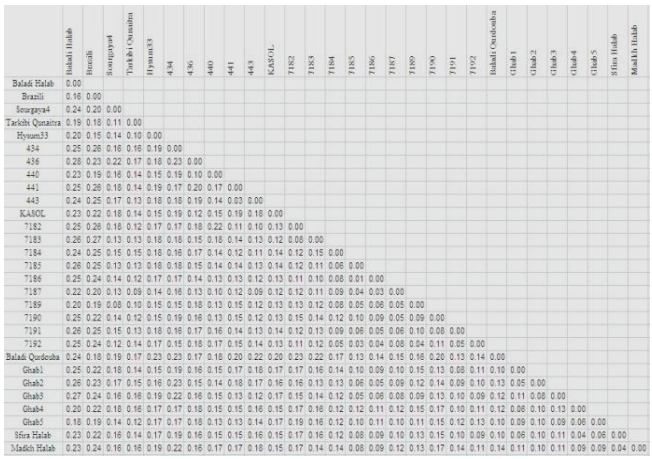
Table 3 -Names and ISSR primer sequences and number of polymorphic lines. Percentage of Disagreement Values (PD

Primers		Sequence (5′-3′)	No. Of Polymorphic lines	% Polymorphic lines	Genotype with single bands	PIC	EMR	MI
A1	CACACACACACARR	6	0	0	-	0.00	0.00	0.00
A4	CACACACACACARY	8	2	25	-	0.05	0.33	0.02
A7	CACACACACACARM	6	0	0	-	0.00	0.00	0.00
A10	CACACACACACARK	6	0	0	-	0.00	0.00	0.00
A13	CACACACACACARS	4	0	0	-	0.00	0.00	0.00
A16	CACACACACACAR	6	2	33	-	0.08	0.44	0.04
A26	CACACACACACAK	3	0	0	-	0.00	0.00	0.00
A31	AGCAGCAGCAGCR	7	1	14	-	0.05	0.10	0.00
A35	AGCAGCAGCAGCY	5	0	0	-	0.00	0.00	0.00
A37	AGCAGCAGCAGCM	6	1	17	7182 (600 bp)	0.01	0.16	0.00
A41	AGCAGCAGCAGCK	6	0	0	-	0.00	0.00	0.00
A44	AGCAGCAGCAGCS	5	0	0	-	0.00	0.00	0.00
B1	CTCTCTCTCTCTCTTG	5	1	20	-	0.04	0.09	0.00
B4	CACACACACACAGG	6	2	33	-	0.09	0.67	0.06
B7	GTGGTGGTGGC	6	1	17	Hysum33 (300-400 bp)	0.01	0.011	0.00
B13	CAACAACAACAACAA	5	0	0	-	0.00	0.00	0.00
B16	GACAGACAGACAGACA	5	2	40	-	0.07	0.50	0.03
C22	AGAGAGAGAGAGAGAGT	5	2	40	Baldihalab (300-400 bp)	0.02	0.67	0.01
C24	CTCTCTCTCTCTCTTG	4	1	25	-	0.06	0.13	0.01
C26	CACACACACACAGG	8	6	75	Ghab 5 (800 bp)	0.14	3.27	0.45
C30	CAACAACAACAACAA	6	2	33	-	0.03	0.36	0.01
164/1	AGAGAGAGAGAGAGAGAGT	3	0	0	-	0.00	0.00	0.00
164/2	AGAGAGAGAGAGAGAGAGC	4	0	0	-	0.00	0.00	0.00
164/3	ACTGACTGACTGACTG	4	2	50	Baladihalab (600-500 bp)	0.04	0.50	0.02
Total		129	25					

Results ranged between 0.01 and 0.27 (average 0.14) for RAPD and between 0.01 and 0.07 (average 0.04) for ISSR. Combined RAPDs and ISSRsdatarevealed PDVs ranging between 0.02 and 0.13 (average 0.07),showing that at least 2% of genetic differences existed between the genotypes (Figure 3). A relatively low correlation (R = 0.30) was obtained between PDVs of RAPD and those of ISSR. Combined RAPD and ISSR PDVs were lower than those of RAPD (Figure 4), but they seemed to be more authentic. They allowed a logical clustering of Ghab genotypes (Ghab 1 to Ghab 5), as well as the Greek genotypes from 7182 to 7192 and those from 434 to 443 (Figure 5). References plants showed high values of PD with sunflower genotypes (0.42 - 0.55 for RAPD and 0.53 – 0.61 for ISSR).

	Baladi Halab	Brazili	Serguyya4	Tarikhi Qunaitra	Hysum33	434	436	440	441	443	KASOL	7182	7183	7184	7185	7186	7187	7189	7190	7191	7192	Baladi Qurdouba	Ghab1	Ghab2	Ghab3	Ghab4	Ghab5	Sfira Halab	Madikh Halab
Baladi Halab	0.00																												
Brazili	0.16	0.00																											
Serguyya4	0.24	0.20	0.00																										
Tarikhi Qunaitra	0.19	0.18	0.11	0.00																									
Hysum33	0.20	0.15	0.14	0.10	0.00																								
434	0.25	0.26	0.16	0.16	0.19	0.00																							
436	0.28	0.23	0.22	0.17	0.18	0.23	0.00																						
440	0.23	0.19	0.16	0.14	0.15	0.19	0.10	0.00																					
441	0.25	0.26	0.18	0.14	0.19	0.17	0.20	0.17	0.00																				
443	0.24	0.25	0.17	0.13	0.18	0.16	0.19	0.14	0.03	0.00																			
KASOL	0.23	0.22	0.18	0.14	0.15	0.19	0.12	0.15	0.19	0.18	0.00																		
7182	0.25	0.28	0.18	0.12	0.17	0.17	0.18	0.22	0.11	0.10	0.13	0.00																	
7183	0.26	0.27	0.13	0.13	0.18	0.18	0.15	0.18	0.14	0.13	0.12	0.08	0.00																
7184	0.24	0.25	0.15	0.15	0.18	0.16	0.17	0.14	0.12	0.11	0.14	0.12	0.15	0.00															
7185	0.26	0.25	0.13	0.13	0.18	0.18	0.15	0.14	0.14	0.13	0.14	0.12	0.11	0.08	0.00														
7186	0.25	0.24	0.14	0.12	0.17	0.17	0.14	0.13	0.13	0.12	0.13	0.11	0.10	0.08	0.01	0.00													
7187	0.22	0.20	0.13	0.09	0.14	0.16	0.13	0.10	0.12	0.09	0.12	0.12	0.11	0.09	0.04	0.03	0.00												
7189	0.20	0.19	0.08	0.10	0.15	0.15	0.18	0.13	0.15	0.12	0.13	0.13	0.12	0.08	0.05	0.06	0.05	0.00											
7190	0.25	0.22	0.14	0.12	0.15	0.19	0.16	0.13	0.15	0.12	0.13	0.15	0.14	0.12	0.10	0.09	0.05	0.09	0.00										
7191	0.26	0.25	0.15	0.13	0.18	0.16	0.17	0.16	0.14	0.13	0.14	0.12	0.13	0.09	0.06	0.05	0.06	0.10	0.08	0.00									
7192	0.25	0.24	0.12	0.14	0.17	0.15	0.18	0.17	0.15	0.14	0.13	0.11	0.12	0.05	0.03	0.04	0.08	0.04	0.11	0.05	0.00								
Baladi Qurdouba	0.24	0.18	0.19	0.17	0.23	0.23	0.17	0.18	0.20	0.22	0.20	0.23	0.22	0.17	0.13	0.14	0.15	0.16	0.20	0.13	0.14	0.00							
Ghab1	0.25	0.22	0.18	0.14	0.15	0.19	0.16	0.15	0.17	0.18	0.17	0.17	0.16	0.14	0.10	0.09	0.10	0.15	0.13	0.08	0.11	0.10	0.00						
Ghab2	0.26	0.23	0.17	0.15	0.16	0.23	0.15	0.14	0.18	0.17	0.16	0.16	0.13	0.13	0.06	0.05	0.09	0.12	0.14	0.09	0.10	0.13	0.05	0.00					
Ghab3	0.27	0.24	0.16	0.16	0.19	0.22	0.16	0.15	0.13	0.12	0.17	0.15	0.14	0.12	0.05	0.06	0.08	0.09	0.13	0.10	0.09	0.12	0.11	0.08	0.00				
Ghab4	0.20	0.22	0.18	0.16	0.17	0.17	0.18	0.15	0.15	0.16	0.15	0.17	0.16	0.12	0.12	0.11	0.12	0.15	0.17	0.10	0.11	0.12	0.06	0.10	0.13	0.00			
Ghab5	0.18	0.19	0.14	0.12	0.17	0.17	0.18	0.13	0.13	0.14	0.17	0.19	0.16	0.12	0.10	0.11	0.10	0.11	0.15	0.12	0.13	0.10	0.09	0.10	0.09	0.06	0.00		
Sfira Halab	0.23	0.22	0.16	0.14	0.17	0.19	0.16	0.15	0.15	0.16	0.15	0.17	0.16	0.12	0.08	0.09	0.10	0.13	0.15	0.10	0.09	0.10	0.08	0.10	0.11	0.04	0.06	0.00	
Madikh Halab	0.23	0.24	0.16	0.16	0.19	0.22	0.16	0.17	0.17	0.18	0.15	0.17	0.14	0.14	0.08	0.09	0.12	0.13	0.17	0.14	0.11	0.14	0.11	0.10	0.11	0.09	0.09	0.04	0.00

Fig. 3Percent disagreement values (PDVs) for the sunflower genotypes produced by the 13 polymorphic RAPD primers using UPGMA routine of STATISTICA program.



	Baladi Halab	Brazili	Sourgaya4	Tarkibi Qunaitra	Hysum33	434	436	440	441	443	KASOL	7182	7183	7184	7185	7186	7187	7189	7190	7191	7192	Baladi Qurdouba	Ghab1	Ghab2	Ghab3	Ghab4	Ghab5	Sfira Halab	Madkh Halab
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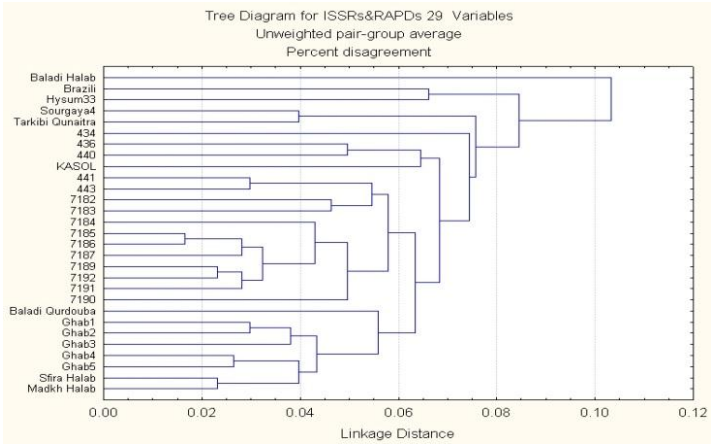
Hysum33	0.20	0.15	0.14	0.10	0.00																								
434	0.25	0.26	0.16	0.16	0.19	0.00																							
436	0.28	0.23	0.22	0.17	0.18	0.23	0.00																						
440	0.23	0.19	0.16	0.14	0.15	0.19	0.10	0.00																					
441	0.25	0.26	0.18	0.14	0.19	0.17	0.20	0.17	0.00																				
443	0.24	0.25	0.17	0.13	0.18	0.18	0.19	0.14	0.03	0.00																			
KASOL	0.23	0.22	0.18	0.14	0.15	0.19	0.12	0.15	0.19	0.18	0.00																		
7182	0.25	0.26	0.18	0.12	0.17	0.17	0.18	0.22	0.11	0.10	0.13	0.00																	
7183	0.26	0.27	0.13	0.13	0.18	0.18	0.15	0.16	0.14	0.13	0.12	0.08	0.00																
7184	0.24	0.25	0.15	0.15	0.18	0.16	0.17	0.14	0.12	0.11	0.14	0.12	0.15	0.00															
7185	0.26	0.25	0.13	0.13	0.18	0.18	0.15	0.14	0.14	0.13	0.14	0.12	0.11	0.06	0.00														
7186	0.25	0.24	0.14	0.12	0.17	0.17	0.14	0.13	0.13	0.12	0.13	0.11	0.10	0.08	0.01	0.00													
7187	0.22	0.20	0.13	0.09	0.14	0.16	0.13	0.10	0.12	0.09	0.12	0.12	0.11	0.09	0.04	0.03	0.00												
7189	0.20	0.19	0.08	0.10	0.15	0.15	0.18	0.13	0.15	0.12	0.13	0.13	0.12	0.08	0.05	0.06	0.05	0.00											
7190	0.25	0.22	0.14	0.12	0.15	0.19	0.16	0.13	0.15	0.12	0.13	0.15	0.14	0.12	0.10	0.09	0.05	0.09	0.00										
7191	0.26	0.25	0.15	0.13	0.18	0.16	0.17	0.16	0.14	0.13	0.14	0.12	0.13	0.09	0.06	0.05	0.06	0.10	0.08	0.00									
7192	0.25	0.24	0.12	0.14	0.17	0.15	0.18	0.17	0.15	0.14	0.13	0.11	0.12	0.05	0.03	0.04	0.08	0.04	0.11	0.05	0.00								
Baladi Qurdouba	0.24	0.18	0.19	0.17	0.23	0.23	0.17	0.18	0.20	0.22	0.20	0.23	0.22	0.17	0.13	0.14	0.15	0.16	0.20	0.13	0.14	0.00							
Ghab1	0.25	0.22	0.18	0.14	0.15	0.19	0.16	0.15	0.17	0.16	0.17	0.17	0.16	0.14	0.10	0.09	0.10	0.15	0.13	0.08	0.11	0.10	0.00						
Ghab2	0.26	0.23	0.17	0.15	0.16	0.23	0.15	0.14	0.18	0.17	0.16	0.16	0.13	0.13	0.06	0.05	0.09	0.12	0.14	0.09	0.10	0.13	0.05	0.00					
Ghab3	0.27	0.24	0.16	0.16	0.19	0.22	0.16	0.15	0.13	0.12	0.17	0.15	0.14	0.12	0.05	0.06	0.08	0.09	0.13	0.10	0.09	0.12	0.11	0.08	0.00				
Ghab4	0.20	0.22	0.18	0.16	0.17	0.17	0.18	0.15	0.15	0.16	0.15	0.17	0.16	0.12	0.12	0.11	0.12	0.15	0.17	0.10	0.11	0.12	0.06	0.10	0.13	0.00			
Ghab5	0.18	0.19	0.14	0.12	0.17	0.17	0.18	0.13	0.14	0.17	0.19	0.16	0.12	0.10	0.11	0.10	0.11	0.15	0.12	0.13	0.10	0.09	0.10	0.09	0.06	0.00			
Sfira Halab	0.23	0.22	0.16	0.14	0.17	0.19	0.16	0.15	0.15	0.16	0.15	0.17	0.16	0.12	0.08	0.09	0.10	0.13	0.15	0.10	0.09	0.10	0.08	0.10	0.11	0.04	0.06	0.00	
Madkh Halab	0.23	0.24	0.16	0.16	0.19	0.22	0.16	0.17	0.17	0.18	0.15	0.17	0.14	0.14	0.08	0.09	0.12	0.13	0.17	0.14	0.11	0.14	0.11	0.10	0.11	0.09	0.09	0.04	0.00

Fig. 4.Percent disagreement values (PDVs) for the sunflower genotypes produced by the 24 polymorphic ISSR primers using UPGMA routine of STATISTICA program

Fig. 5Cluster analysis based on percent disagreement values of UPGMA, Statisticausing the combined RAPD and ISSR data of sunflower genotypes



Polymorphism evaluation:

PIC values reached 0.31 (RAPD primerR04), with an average of 0.13 (calculated from the total number of primers) (Table 2). However, the highest PIC value calculated on the bases of ISSR markers was only 0.14 (obtained with C26 primer).The average obtained through twenty-four ISSR primers was very low (0.03) (Table 3). A notable similarity was found between PDV and PIC averages for both RAPD and ISSR (respectively 0.14, 0.13 for RAPD and 0.04, 0.03 for ISSR).

The highest EMR and MI were respectively 11 and 3.36, obtained with RAPD primerR04 (Table 2). On the other hand, highest values of EMR and MI were 3.27 and 0.45 for ISSR, obtained with C26 primer (Table3). It is important to note that most of the ISSR primers (14 among 24 primers) gave only monomorphic markers (EMR and MI equal to zero).

3-Discussion:

Twotechniqueswere employed in this study: RAPD, which is commonly used in molecular analyses of many species, including sunflower [15, 16, 17] and ISSR, which is to our knowledge employed for the first time on local sunflower genotypes. One of our objectives was testing the efficiency of ISSR as compared with that of RAPD. The second objective was studying the genetic diversity within the twenty-seven

local and introduced sunflower genotypes (Table 1), and exploring the possibility of utilizing it in a sunflower breeding program based on Marker Assisted Selection (MAS).

The comparison between these two techniques concerned the polymorphism provided by each one and its effect on PDV. For this, PDV, PIC, EMR and MI were calculated on the bases of RAPD and ISSR molecular analyses data.

A remarkable difference existed between PDVs of RAPD and those of ISSR resulting in a relatively low correlation (R = 0.30). Mahmoud and Abdel-Fatah obtained lower correlation between RAPD and ISSR (0.17) using thirteen sunflower genotypes and dissimilarity values [28]. However, higher correlation (0.53) between RAPD and ISSR data was obtained by MirAli*et al*. who studied PDVs on fifty nine samples of *Pyrussyriaca*collected from different geographical regions in Syria[29]. Likewise, Goulao and Oliveira study revealed a high correlation between data obtained from four different techniques (AFLP, RAPD, SSR and ISSR), used to analyze apple genotypes [30]. Baladihalab had the highest PDV among sunflower genotypes; it did not belong to any cluster, which makes it a good parent for breeding. It would be also interesting to verify if these bands were linked to some specific traits. It is important to mention the notable high PDVs obtained between references, plants and sunflower genotypes.

Low correlations between techniques could be due, in part, to the degree of polymorphism provided by them. PIC values confirmed this by being higher with RAPD markers than with ISSRs. Higher PIC values in RAPDsare mainly due to the random annealing of primers, allowing amplification of DNA sequences from all over the genome contrary to the limited specific regions amplified in ISSR.Blanda*et al*. mentioned that the flanking regions for most of the microsatellite (SSRs) are generally highly conserved [31]. For dominant markers, the highest PIC value is limited to 0.5, indicating that an average of 0.13 (obtained with RAPD) expressed a relatively high genetic diversity among sunflower genotypes. EMR and MI results confirmed, once more, that polymorphism was considerably more important with RAPD.

According to these results, ISSR is not recommended as a single method for sunflower fingerprinting. Compared with RAPD, this technique provides low degrees of polymorphism, which makes RAPD more appropriate for this kind of studies. *Iqbal et al*. also reported high degrees of polymorphism between sunflower genotypes in their research using RAPD [5]. However, Wahabi and Bukhari recommended ISSR in determining genetic variation but they didn't compare it with any other technique in their study [32]. Results obtained by Mahdizadeh*et al*. showed that both techniques were suitable for the detection of genetic polymorphism among sesame *M.* phaseolina isolates [33]. Low degrees

of polymorphism between Bulgarian sunflower cultivars were obtained by Hvarleva*et al*. using SSR technique [34]. Wild sunflower genotypes seemed to be more polymorphic when compared with cultivated ones [35]. Techniques like RFLP and AFLP provided numerous polymorphic markers in sunflower and were thus widely used in genetic diversity and mapping [2, 7, 10, 11]. Nevertheless, techniques providing high degree ofmonomorphismshould not be excluded. Although they do not change relationships among genotypes; monomorphic markers have the role to correct the exaggerated values of PDV which could result if only polymorphic markers were used.It is concluded that combining different types of markers is necessaryto overcome the problem of monomorphism and for maximum coverage of a genome.

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