

Estimation the Degree of Diversity for Some Iraqi Wheat Varieties through ISSR, SRAP and RAPID Markers

Abstract

ISSR, SRAP and RAPID markers were used to explain the genetic differences among sixteen Iraqi wheat varieties. 73 primers out of 177 for the used markers were producible and showed clear amplified bands. The degree of genetic diversity, Polymorphism information content (PIC) and Resolving Power (RP) were estimated. All the studied molecular markers were informative and showed good ability to classify the wheat varieties. Total number of polymorphic bands was 134, 221 and 55 for ISSR, SRAP and RAPID respectively. PIC and RP values were 0.259, 0.264 and 0.262 and 9.06, 7.87 and 2.78 respectively. It seems that SRAP marker was the most optimal technique for characterization the Iraqi wheat varieties. The most efficiency primers were Ne ISSR 7 for ISSR; Me4-Em3 and Me4-Em9 primers combination for SRAP; K14, K15 and K19 for RAPID. The results of this study can be used to contribute in putting the core stone of the data base for Iraqi wheat varieties to be used in the future as an indicator for breeding programs.

Keywords: Wheat, Genetic Diversity, ISSR, SRAP, RAPID

Introduction

Wheat (*Triticum aestivum* L.) is one of the most and dominant cereals in the world and Iraq. The grain yield of wheat continued to increase, as a result of new varieties came under cultivation and the improved agricultural husbandry involving the use of chemical fertilizers, however, Iraq still imports tons of wheat every year. During the past several decades, the quality and yield of wheat have been gradually improved by traditional breeding methods. These methods have some restricting factors such as long time required and the limited gene pool that is available for wheat breeders. To increase wheat production in Iraq, it is a pre-requisite that local wheat germplasm should be characterized and classified by using new fast, easy and reliable technologies especially genetic markers (Galovic *et al.*, 2005). Most researches all over the world nowadays depend on using molecular techniques (Repkova *et al.*, 2009). Considerable efforts have been made to improve wheat crop productivity by using biotechnology. The morphological traits are greatly

influenced by the environment, so diversity studies based on morphological traits may not be completely reliable, inaccurate and unreliable. Molecular diversity is independent of environmental influences because it is evaluated by using molecular markers and can be estimated by using DNA from any growth stage (Tatikonda *et al.*, 2009). Genetic diversity can be considered the basis for genetic improvement, and knowledge of germplasm diversity has a significant impact on the improvement of wheat crop plants. According to modern breeding, it is suggested that genetic diversity in wheat is narrowed increasingly. Therefore, in order to broaden the genetic variation in future wheat breeding it is necessary to investigate the genetic diversity in wheat germplasm (Mohammadi and Prasanna, 2003). Using molecular markers for the evaluation of genetic diversity is receiving much attention. Many scientists have investigated the genetic diversity in wheat crop using different molecular markers like Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), and Inter Simple Sequence Repeat (ISSR),..etc. PCR-based genomic polymorphism has been detected in cultivated hexaploid wheat (*Triticum aestivum* L.) by different markers such as microsatellite markers (Hamdalla, 2014, Akkaya and Buyukunal-Bal,2004, and Dograr,2000). Inter Simple Sequence Repeat (ISSR) markers are also useful to detect genetic polymorphism in this species. Furthermore, one of the new types of molecular markers, Sequence-Related Amplified Polymorphism (SRAP) was developed and used in wheat plant genetic studies. The goal of this study is to differentiate among Iraqi wheat varieties using some molecular techniques to explain the genetic differences.

Material and Methods

Plant materials & DNA extraction

Sixteen Iraqi wheat varieties were obtained from Iraqi Ministry of Agriculture (table 1). These experiments were conducted at Crop Research Laboratory / Dept. of Agronomy and Horticulture/ University of Nebraska/

USA. 100 mg of wheat seedling leaves were put in 2 ml tubes and kept in -80°C freezer. Plant leaves were dried by putting them in freeze dryer (Vir Tis, Model# FM 25 XL-70) for twenty four hours. A metal ball was put in each tube contained the lyophilized tissue. The leaf tissues were grinded by tissue lyzer II (Qiagen, Cat # 85300) at speed 39 S/min for 3min. The extraction protocol was Adapted from (Zhanguo Xin & Junping Chen, 2012; Xin, and Junping C. 2012)

Table 1 the pedigree of foreign wheat varieties

Symbol	Genotype	Pedigree
A	Tamooz 2	Mexipak / Saberbeg → (radiation)
B	Iraq	
C	Iba 99	Ures/Bow s / 3/Jup/Biy s /ures
D	Rabee	Saberbeg / Hindia HDE831
E	Um Rabee	Jori69 / Haw
X	Buhooth 22	-
G	Tamooz 3	Mexipak /Saberbeg// Abu-ghurib →(radiation)
H	Iba 95	Veery S
I	Dore 29	
J	Latifia	Australian line / Aras → (selection)
Y	Dore 85	
S	Hashimia	Ecarda
O	Fatih	-
P	Abu Graib	Ajeeba / Inia 66R // Meico24 → (selection)
M	Dijla	F ₂ materials derived from a commercial wheat breeding program, USA→ (radiation)
N	Furat	F ₂ materials derived from a commercial wheat breeding program, USA→ (radiation)

Primer selection

Sixteen Iraqi varieties were screened with each of ISSR, SRAP, and RAPD techniques. 20 primers out of 23 ISSR were producible and showed clear amplified bands. For SRAP, 54 primers were screened, 28 of them produced amplified bands, whereas 25 RAPD primers out of 100 ones were able to produce polymorphic bands.

ISSR primers

Table 2 ISSR primers

Primer symbol	primer	Sequences	Primer symbol	primers	Sequences
3	Ne ISSR 20	GATCTTCTTC TTCTTCTTCT TCT	7	Ne ISSR 14	GATCCGGCGG CGGCGGCGG
4	Ne ISSR 10	CCCGGATCC GAGAGAGAG AGA	10	Ne ISSR 17	GATCCAGCAG CAGCAGCAG
5	Ne ISSR 9	GCGATTATT ATTATTATT	12	Ne ISSR 15	GATCTGGTGG TGGTGGTGG
8	Ne ISSR 7	AGCAGCAGC AGCAGCGA	16	Ne ISSR 21	GATCTCTGTCT GTCTGTCTGTC TG
6	Ne ISSR 18	GATCATCAT CATCATCATC	18	Ne ISSR 22	GATCAGTGAG TGAGTGAGTG AGTG
1	Ne ISSR 8	GGCGGCGGC GGCTA	19	Ne ISSR 12	CCCGGATCCG TGTGTGTGTG T
2	Ne ISSR 19	GATCTTGTTG TTGTTGTTG	22	Ne ISSR 11	CCCGGATCCC TCTCTCTCTCT

9	Ne ISSR 13	CCCGGATCC CACACACAC ACA	20	Ne ISSR 3	GAGAGAGAGA GAGAGATC
11	Ne ISSR 16	GATCGAGGA GGAGGAGGA G	14	Ne ISSR 6	AAGAAGAAGA AGAAGTG
13	Ne ISSR 4	CACACACAC ACACACAGT	15	Ne ISSR 5	AAGAAGAAGA AGAAGGC

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81 SRAP Primer

82 This technique depends on combination between two types of
83 primers. Table -a shows the sequence of primers that were producible,
84 and table -b shows the combinations that produced polymorphic bands.

85 Table 3 -a SRAP primers

Me1	TGA GTC CAA ACCGGA TA	Em1	GAC TGC GTA CGA ATT AAT
Me2	TGA GTC CAA ACC GGA GC	Em2	GAC TGC GTA CGA ATT TGC
Me3	TGA GTC CAA ACC GGA AT	Em3	GAC TGC GTA CGA ATT GAC
Me4	TGA GTC CAA ACC GGA CC	Em4	GAC TGC GTA CGA ATT TGA
		Em5	GAC TGC GTA CGA ATT AAC
		Em6	GAC TGC GTA CGA ATT GCA
		Em7	GAC TGC GTA CGA ATT CAA
		Em8	GAC TGC GTA CGA ATT CAC
		Em9	GAC TGC GTA CGA ATT CAG
		Em10	GAC TGC GTA CGA ATT CAT
		Em11	GAC TGC GTA CGA ATT CTA
		Em12	GAC TGC GTA CGA ATT CTC
		Em13	GAC TGC GTA CGA ATT CTG
		Em14	GAC TGC GTA CGA ATT CTT
		Em15	GAC TGC GTA CGA ATT GAT

		Em16	GAC TGC GTA CGA ATT GTC
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Table 3 -b Combination of SRAP primers used in the study

Symbol of combination	me	Em	Symbol of combination	me	Em
31	me 2	em 1	52	me 4	em 10
32	me 2	em 2	53	me 4	em 11
33	me 3	em 3	54	me 4	em 12
34	me 3	em 4	8	me 1	em8
43	me 3	em 1	9	me 1	em9
44	me 3	em 2	12	me 1	em12
45	me 4	em 3	19	me 2	em4
46	me 4	em 4	21	me 2	em6
48	me 4	em 6	22	me 2	em7
49	me 4	em 7	23	me 2	em8
50	me 4	em 8	26	me 2	em11
51	me 4	em 9	27	me 2	em12
25	me 2	em10	4	me 1	em4
3	me 1	em3	15	me 1	em16

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89

RAPD Primers

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Table 4 RAPD Primers

Primer	Sequence	Primer	Sequence
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91	OP K- 16	GAGCGTCGAA	OP L- 12	GGGCGGTACT
92				
93	OP K- 15	CTCCTGCCAA	OP L- 13	ACCGCCTGCT
94				
95	OP K- 8	GAACACTGGG	OP M- 9	GTCTTGCGGA
96				
	OP K- 9	CCCTACCGAC	OP M- 8	TCTGTTCCCC
	OP K- 10	GTGCAACGTG	OP M- 6	CTGGGCAACT
	OP K- 6	CACCTTTCCC	OP M- 11	GTCCACTGTG
	OP K- 12	TGGCCCTCAC	OP M- 16	GTAACCAGCC
	OP K- 11	AATGCCCCAG	OP M- 10	TCTGGCGCAC
	OP K- 14	CCCGCTACAC	OP O- 9	TCCCACGCAA
	OP K- 20	GTGTCGCGAG	OP N- 05	ACTGAACGCC
	OP K- 19	CACAGGCGGA	OP N- 7	CAGCCCAGAG
	OP L-6	GAGGGAAGAG	OP N-8	ACCTCAGCTC
	OP L-9	TGCGAGAGTC		

Polymerase Chain Reaction

The PCR mixture (25 µl total) consisted of 50 mM KCl and 10 mM Tris-HCl (pH 8.8), 2 mM MgCl₂, 125 mM of dNTP, 50 ng of each primer, 1.0 unit of Taq DNA polymerase, and 60 ng of genomic DNA.

Amplification was carried out in C 1000 Touch™ Thermal Cycler (Bio-Rad) using a program that consisted of initial denaturation for 3 min. at 95°C, followed by 34 cycle of 1 min. at 94 °C, 1min at 45 to 55 ° (depending on the primers), and 1 min. at 72°C, and final extension for 10 min. The amplified reaction mixtures were stored at 4°C until they were gel- fractionated on either polyacrylamide gel, or agarose gel (depending on the molecular technique).

Electrophoreses

The amplified reaction mixtures of SSR, SRAP, and ISSR were gel-fractionated on 12% (w/v) polyacrylamide gel (37crylamide: 1bis-acrrylamide) in 1X DGGE buffer (40 mM Tris-HCl, 20mM sodium acetate, 1 mM EDTA) in vertical gel apparatus (Hofer Scientific Instrument, SE600) at 300V for 3 h. The buffer temperature was controlled by a circulating bath with the temperature set to 20°C. Gels were stained in ethidium bromide (1mg/mL) for 20 min, destained in deionized water for 1h, and then photographed by means of Gel Doc 2000 (Bio-Red).

The amplified products of RAPD primers Electrophoresed in 1% agarose gel with 1X TBE buffer, and 2 µl of ethidium bromide using 70 volts. The amplified markers were photographed by Gel Doc 2000 (Bio-Red).

Statistical Analysis

Data obtained from experiments of media type were collected and scored for the presence (1) or absence (0) of amplified bands. The genetic similarity coefficients for each pairwise of varieties were calculated by using software R (version 3. 0. 1 (2013-05-16) Copyright (C) 2013 The R Foundation for Statistical Computing Platform: i386-w64-mingw32/i38).

Resolving power (RP) was calculated according to the following equation;

$$RP = \sum 1 - (2 * (0.5 - f))$$

Where f is the frequencies.

Polymorphism information content (PIC) values were obtained using the following formula: $PIC = 1 - \sum P_{ij}^2$

Where P_{ij} is the frequency of jth allele of ith locus, summed across all the alleles for the locus over all lines. A PIC value > 0.5 is considered as being highly informative marker while $0.5 > PIC > 0.25$ is an informative marker, and PIC is 0.25 a slightly informative marker (Botstein et al., 1980)

Results and Discussion

ISSR Marker

Twenty ISSR primers yielded in total 167 bands of which 134 were polymorphic (table 5) with average percentage of polymorphism 80.24%. Amplified bands per primer ranged from 2 (primer 11) to 26 bands (primer 8) with an average of 8.4 bands. The level of polymorphism per primer ranged from 50 up to 100%. Primer 8 showed the highest number of amplified bands with the highest number of polymorphic bands. PIC values ranged from very low value of primer 4 (0.059) to high values (0.467 and 0.420) of primers 2 and 15 respectively, and the average was 0.259, referring that this technique is informative in genetic diversity (Botstein 1980). RP value ranged from 1.13 to 33.00 with an average of 9.06 per primer. The highest RP was recorded by the primer 8 followed by the primer 18 referring to their importance in genetic diversity, because of RP positive correlation with the total amplified bands (Najaphy, et. al. 2011).

Primers with di-nucleotide repeat produced low level of polymorphism in most primers used in this study in comparison with tri- nucleotide and tetra-nucleotide primers specially the di-nucleotide with GA repeats. tri- nucleotide

primers with repeats AGC and ATC showed the highest number of polymorphic bands. These results are in agreement with those of El-Assal and Gaber,(2012); Akkaya et al. (1992); Sofalian, et al. (2008) who confirmed that di-nucleotide repeat primers were found at lower polymorphic levels.

According to PIC and RP values, the results revealed efficiency of primer 8 which has consist of tri-nucleotides repeats (TTG & AGC) . In spite of that tri-nucleotides repeats show in low frequency as compare to di-nucleotides (Blair & et al,1999; Joshi & et al,2000), TTG & AGC seem useful in characterization the Iraqi wheat varieties.

Table 5 ISSR primers with Iraqi varieties

Prim .	No of Alle l	monomo r.	Polymorphi c bands	polymorph. %	PIC	RP
3	3	0	3	100.00	0.385	2.75
4	4	2	2	50.00	0.059	4.25
5	5	0	5	100.00	0.195	1.13
8	26	5	21	80.77	0.296	33.00
6	17	2	15	88.24	0.281	16.13
1	4	0	4	100.00	0.301	3.50
2	4	0	4	100.00	0.467	3.88
9	4	0	4	100.00	0.232	5.13
11	2	1	1	50.00	0.152	3.63

13	3	0	3	100.00	0.318	1.25
15	7	0	7	100.00	0.420	6.00
7	9	3	6	66.67	0.213	14.38
10	7	0	7	100.00	0.366	7.50
12	4	0	4	100.00	0.361	3.63
16	14	5	9	64.29	0.167	18.13
18	15	6	9	60.00	0.227	20.63
19	7	1	6	85.71	0.219	4.75
22	6	1	5	83.33	0.247	7.00
20	9	4	5	55.56	0.158	11.75
14	17	3	14	82.35	0.108	12.75
Sum	167	33	134	80.24		181.13
Aver .	8.35	1.65	6.7	80.24	0.259	9.06

164

165 **SRAP**

166 The analysis of the 16 local varieties with 28 SRAP primer combinations
167 identified a total of 248 bands (table 6), among them 221 were polymorphic
168 (89.56%). Between 3 and 19 bands were amplified per primer combination
169 with an average of 8.86 bands. The number of polymorphic bands for each
170 primer combination varied from 3 to 17 with an average of 7.9. Primer
171 combination 43 appeared with the highest number of bands and all of them
172 were polymorphic, followed by the two primer combinations 31 and 49 which

yielded in 17 bands for each, both of them were polymorphic 100%. Primer combination number 23 recorded very low value of PIC, but the values in most of other primers were high and reliable. The highest value was recorded by primer combination number 43 (0.403). The average was 0.264 meaning that this is also informative and reliable in explaining genetic diversity and calculating the distances between Iraqi wheat varieties. RP ranged from 0.63 (primer combination 32) to 19.00 (primer combination 48) with average 7.87. The results revealed that primers combination 43 followed by 49 and 31 were useful to characterize the Iraqi wheat varieties due to them scores of the produced bands, polymorphic bands, RP and PIC.

Table 6 SRAP primer combination with Iraqi varieties

Markers	No of Allel	monomor.	polymorph.	%	RP	PIC
31	17	0	17	100.00	9.88	0.353
32	3	0	3	100.00	0.63	0.185
33	4	1	3	75.00	2.88	0.178
34	5	0	5	100.00	1.13	0.195
43	19	0	19	100.00	16.88	0.403
44	11	3	8	72.73	14.63	0.224
45	8	1	7	87.50	8.38	0.222
46	7	1	6	85.71	6.75	0.234
48	15	1	14	93.33	19.00	0.363
49	17	0	17	100.00	11.63	0.328
50	14	4	10	71.43	18.00	0.208
51	8	2	6	75.00	9.88	0.277
52	7	1	6	85.71	7.25	0.368
53	15	0	15	100.00	9.88	0.329
54	12	1	11	91.67	12.63	0.309
8	7	0	7	100.00	3.88	0.242
9	7	0	7	100.00	5.00	0.205
12	6	0	6	100.00	4.50	0.372
19	9	2	7	77.78	8.25	0.238

21	5	0	5	100.00	4.50	0.388
22	6	2	4	66.67	6.25	0.255
23	6	3	3	50.00	8.00	0.076
26	8	2	6	75.00	7.00	0.135
27	4	1	3	75.00	5.25	0.355
25	7	1	6	85.71	3.75	0.188
3	6	1	5	83.33	6.88	0.205
4	11	0	11	100.00	5.63	0.265
15	4	0	4	100.00	2.00	0.305
SUM	248	27	221	87.56	220.25	
	8.857	0.964	7.893	87.56	7.87	0.264

186

187 \ RAPD

188 Twenty five RAPD primers were chosen as they yielded polymorphic
189 bands. In total 59 bands were amplified and 55 of them were polymorphic
190 with an average of 94% (table 7). The number of polymorphic bands per
191 primer ranged from 1 to 4 with an average of 2.2 bands. The level of
192 polymorphism per primer was mostly 100%, except for the primers L12 and
193 M10; it was 50% for each. Primers: k9, k14, k15, and k16 showed higher
194 number of amplified bands (4), all of them were polymorphic. The highest
195 value of PIC was 0.492, recorded by the primers K10 and L9 followed by
196 other high values recorded by primers M11, L9, and K15. The average of PIC
197 values was 0.262. These values mean that this technique is informative and
198 can be used reliably in genetic diversity clustering. RAPD primers, in
199 general, showed less values of RP than other techniques, as the average was
200 2.85, the maximum value was 7.00 (K16) and the minimum value was 0.50
201 (O9). Primer K15, K14 and K 19 own best combination primers of
202 polymorphism, RP and PIC values, therefore they look optimal primers for
203 characterization the Iraqi wheat varieties. However, the Rapid technique is not
204 efficient as compare with other techniques since it random. The current results
205 supported this fact since the Rapid technique own less values of RP as
206 compare to other techniques.

Table 7 RAPD primers with Iraqi varieties

Primers	No of Alleles	monomorphic	Polymorphic bands	Pol. %	RP	PIC
OP N-05	1	0	1	100	0.13	0.117
OP K-16	4	0	4	100	7.00	0.219
OP K-15	4	0	4	100	5.50	0.426
OP K-8	3	0	3	100	2.13	0.185
OP K-9	4	0	4	100	2.13	0.189
OP K-10	1	0	1	100	0.88	0.492
OP K-6	2	0	2	100	3.75	0.117
OP K-12	2	0	2	100	1.88	0.262
OP K-11	3	0	3	100	3.88	0.117
OP K-14	4	0	4	100	4.00	0.336
OP K-20	3	0	3	100	3.25	0.328
OP K-19	3	0	3	100	4.13	0.393
OP M-9	1	0	1	100	1.38	0.430
OP L-6	3	0	3	100	3.25	0.318
OP L-9	1	0	1	100	1.13	0.492
OP L-	3	0	3	100	5.63	0.117

13						
OP L-12	2	1	1	50	3.75	0.109
OP M-8	2	0	2	100	3.75	0.117
OP M-6	2	0	2	100	3.75	0.117
OP M-11	1	0	1	100	1.25	0.469
OP M-16	2	0	2	100	1.38	0.355
OP N-07	1	0	1	100	1.88	0.117
OP N-08	2	0	2	100	2.00	0.117
OP O-9	1	0	1	100	0.50	0.375
OP M-10	2	1	1	50	2.88	0.246
Sum	57	2	55	2400	71.13	6.562
	2.28	0.08	2.2	96.49 1	2.85	0.262

208

209 Number of Bands per Varieties

210 Table 8 reveals the differences among the wheat varieties according to the
 211 number of bands they produced as a result of scoring with primers of different
 212 molecular techniques. Variety H produced the highest number of band among
 213 Iraqi varieties (154 bands with percent 62.28) when they were scored by ISSR
 214 primers. In SRAP technique variety H achieved 67.74 percent of all primer
 215 combination used in this technique. It produced 104 bands. In RAPD
 216 technique, H variety also gave the highest number of bands (48 bands with
 217 percent 81.36%). There were other more varieties that had high number of
 218 bands from the last three techniques such as variety A and N. Varieties that

produced higher number of band than other are consider more important for researchers in breeding programs.

Table 8 Number and percentage of bands per Varieties

V	ISSR		SRAP		RAPD		Total Bands	Average Bands
	No	%	No	%	No	%		
A	94	56.29	148.00	59.68	43.00	72.88	285	95
B	98	58.68	86.00	34.68	37.00	62.71	221	74
C	91	54.49	78.00	31.45	39.00	66.10	208	69
D	99	59.28	120.00	48.39	40.00	67.80	259	86
E	79	47.31	119.00	47.98	33.00	55.93	231	77
G	98	58.68	126.00	50.81	44.00	74.58	268	89
H	104	62.28	168.00	67.74	48.00	81.36	320	107
I	79	47.31	108.00	43.55	33.00	55.93	220	73
J	90	53.89	99.00	39.92	41.00	69.49	230	77
M	84	50.30	72.00	29.03	15.00	25.42	171	57
N	92	55.09	147.00	59.27	46.00	77.97	285	95
O	85	50.90	80.00	32.26	24.00	40.68	189	63
P	82	49.10	86.00	34.68	37.00	62.71	205	68
S	99	59.28	107.00	43.15	41.00	69.49	247	82
X	91	54.49	102.00	41.13	41.00	69.49	234	78
Y	84	50.30	116.00	46.77	39.00	66.10	239	80

Genetic Diversity

ISSR

The data clustered into three main groups; first group included four varieties (G, H, N, and A). The similarity among them ranged from 0.7108, between N and H varieties, to 0.7952, between A and N varieties. Second group contained three sub clusters each of them contained two varieties. The highest value of coefficient similarity was 0.9277, between S and D varieties, followed by the similarity coefficient between I and E varieties (0.8434), then by Y and O varieties (0.7892). Third group contained six varieties. J and C formed a separate sub cluster, the similarity coefficient between them was 0.8855, and each of others was alone in a separate cluster. The similarity coefficients between each pair of them were 0.6687 between M and X, 0.6627 between M and B, 0.7108 between M and P, 0.698795 between M and J,

0.789157 between X and B, 0.813253 between X and P, 0.825301 between X and J, 0.831325 between B and P, 0.831325 between B and J, and 0.855422 between P and J.

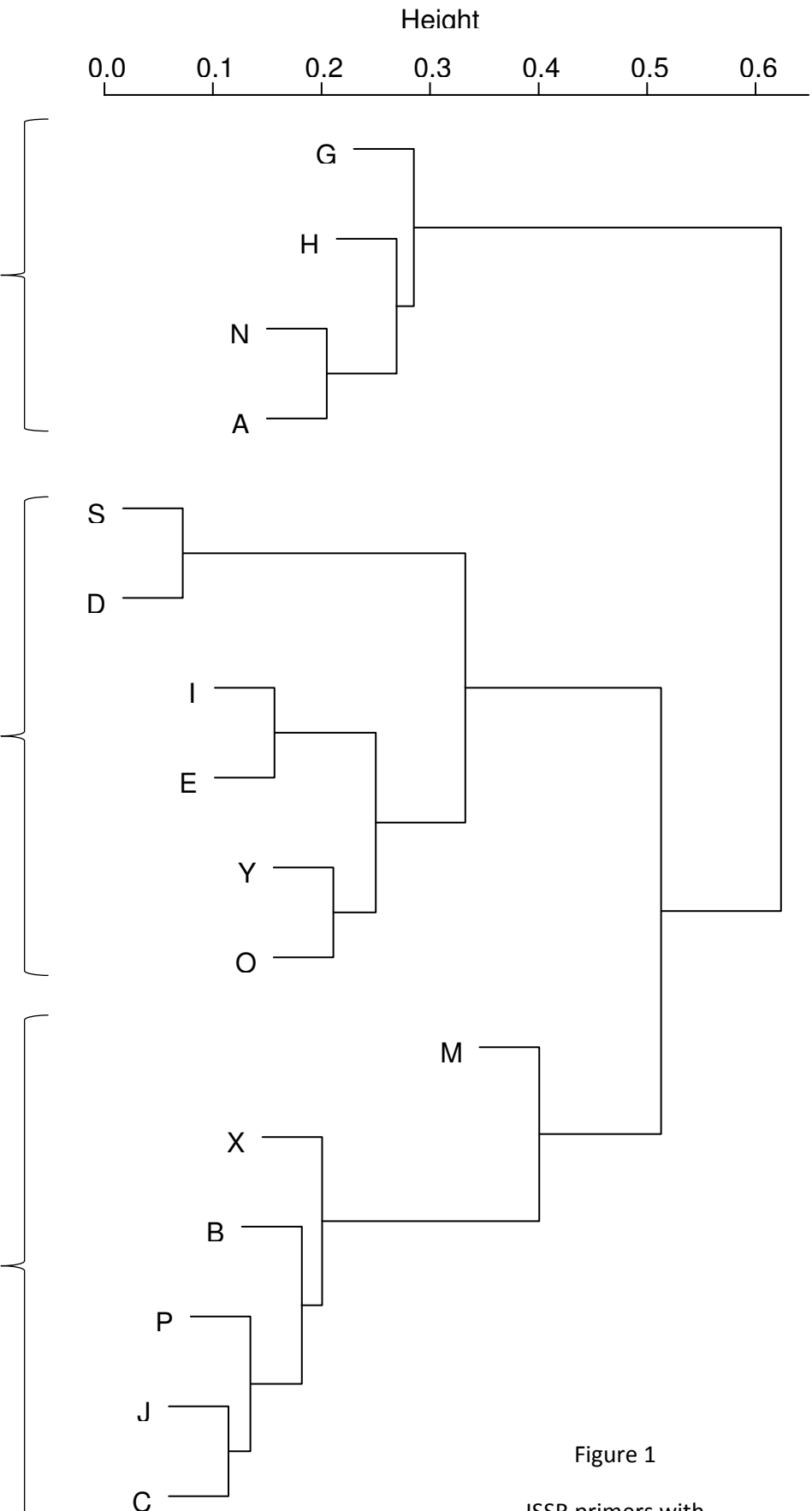
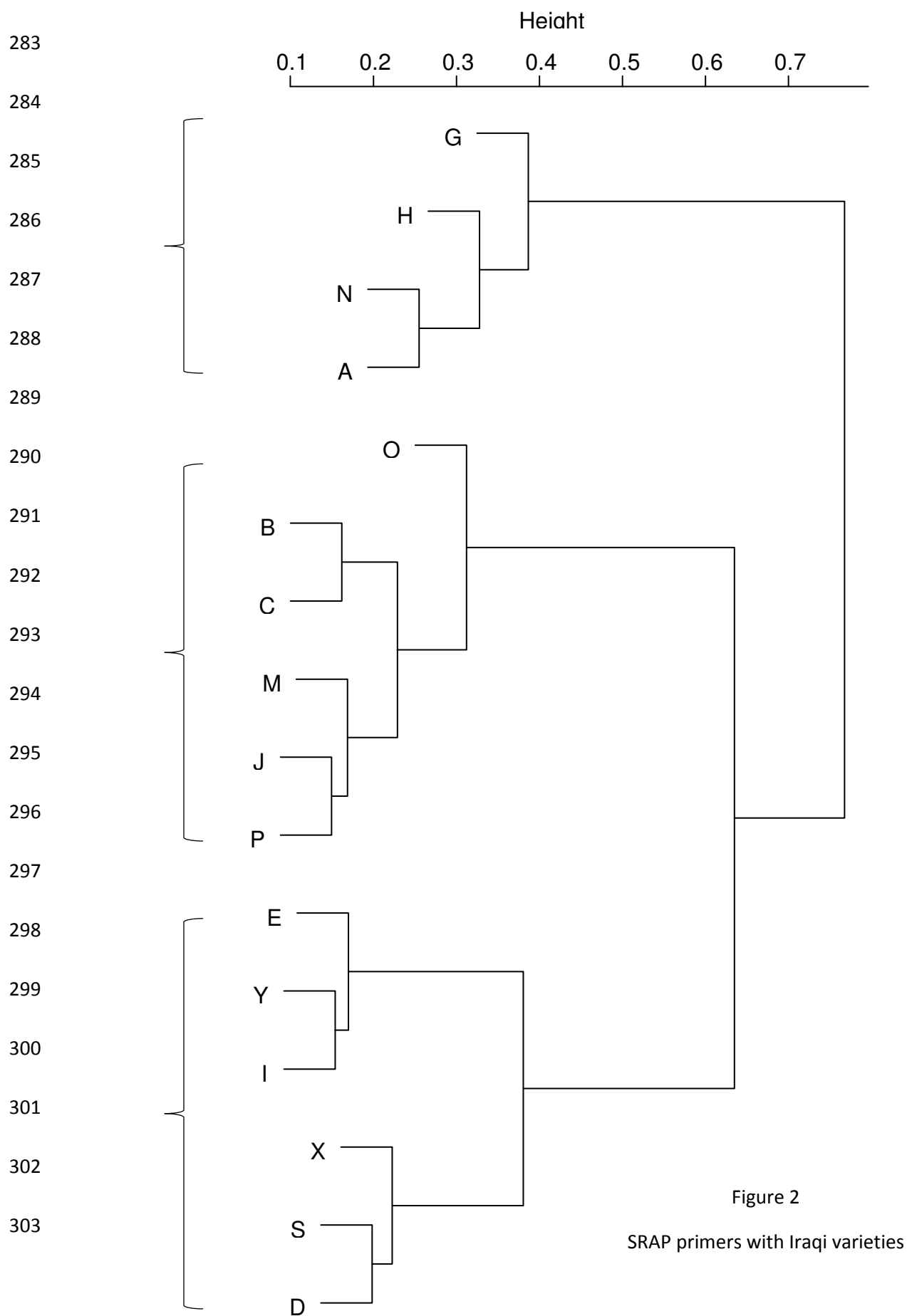


Figure 1

ICSP primers with

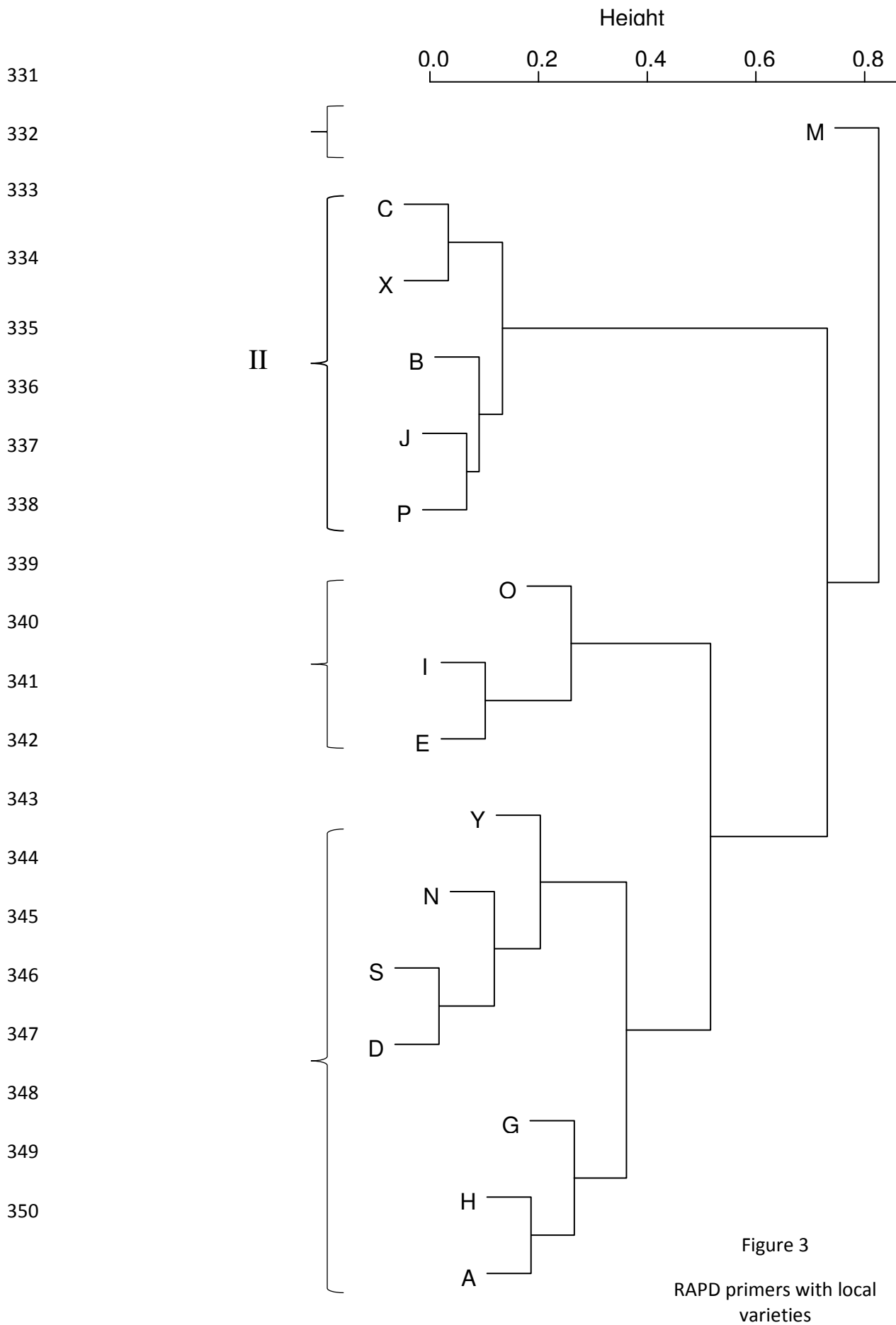
SRAP

Scoring twenty eight SRAP primer combinations showed similarity among sixteen Iraqi wheat varieties ranged from 0.498 to 0.850. The dendrogram (figure 2) revealed three major clusters of wheat varieties. Cluster one contained four varieties (G, H, N, and A). The similarity coefficient between N and A, the highest value among varieties in this cluster, was 0.745. The other coefficients among them were 0.636 between G and H varieties, 0.640 between G and N varieties, 0.660 between G and A varieties, and 0.672 between H and N varieties. Cluster two contained six varieties divided into three sub clusters. O represented a sub cluster, while B and C presented second sub cluster with similarity coefficient about 0.838. Third sub cluster included M, J, and P. The similarity coefficients between them were 0.850 between M and J varieties, 0.822 between M and P, and 0.850 between J and P. Cluster three included two sub clusters, first one included E, Y, and I. The similarity coefficients between them were 0.834 between E and Y varieties, 0.834 between E and I, and 0.846 between Y and Y varieties. The last sub cluster contained X, S, and D. Similarity coefficient between X and S varieties was 0.802, and between X and D was 0.765, whereas between S and D was 0.802.



RAPD

Similarity coefficient among sixteen Iraqi varieties depending on twenty seven RAPD primers ranged from 0.339 to 0.983. Cluster dendrogram classified the evaluated varieties into four clusters. The variety M alone formed the first cluster having less similarity with each of other varieties. Second cluster involved five varieties divided into two sub clusters. First sub cluster contained C and X varieties with similarity coefficient 0.966 between them. Second sub cluster contained three members B, J and P. The highest similarity coefficient among them was 0.932 between the each pairwise of J and P; B and P varieties, followed by B and J. Third cluster included O, I, and E. The similarity coefficients between each pairwise were 0.780 between O and I varieties, 0.780 between O and E varieties, and 0.898 between I and E. Fourth cluster contained two sub clusters. First sub cluster contained four varieties (Y, N, S, and D). The highest value of similarity coefficient among all varieties (0.983) was between S and D varieties. Similarity coefficient between other members of this sub cluster were 0.780 between Y and N varieties, 0.864 between Y and S varieties, and 0.915 between Y and S. G, H , and A formed the last sub cluster. Similarity coefficient between G and H variety was 0.797 between G and H varieties, 0.712 between G and A, finally 0.814 between H and A.



Discussion

The rapidly developing technology of DNA markers helps to open a new real possibility for evolving functional markers as reliable genetic markers for genetic improvement. Varieties H, A and N were the most responsive genotypes to the used primers due to own highest numbers of polymorphism bands (Table 8). Some varieties remain together across the produced dendograms that have been built on various markers. The varieties G and H , S and D , J and P were stayed in same clusters across all markers. While I,E and y; M,B and C; and N and A gathered in same cluster across ISSR and SRAP markers. That would be useful for breeders in breeding schemes in term of developing the local varieties (Wang et al., 2009). All the studied molecular markers were informative and showed good ability to classify the wheat varieties. Table 9 shows some criteria which can be depended to compare among these markers. According to total number of the produced bands, Average number of polymorphic bands per primer, RP and PIC values, SRAP marker can be the suitable technique for characterization the Iraqi wheat varieties. Finally, the information resulted from this study can be used to contribute in putting the core stone of the data base for Iraqi wheat varieties to be used in the future as an indicator for breeding programs because the exclusive use of local genotypes makes the work difficult for the breeder (Hamdalla,2014), so should interest in introduction of elite and new genetic materials to constantly raise productivity and quality of the characteristics for the local genotypes.

Table 9. Comparative of various molecular markers in evaluating the genetic diversity of the studied local wheat varieties.

Primers	ISSR	SRAP	RAPD
Number of Total Primers	23	54	100
Number of primers amplified	20	28	25
Total number of polymorphic bands	134	221	55

Total number of monomorphic bands	33	27	4
Total number of bands	167	248	59
Average percentage of polymorphism (%)	80.24	87.56	93.22
Average number of polymorphic bands/primer	6.7	7.89	2.03
Average RP	9.06	7.87	2.78
Average MRP	0.28	0.19	0.14
Average PIC	0.259	0.264	0.262

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