Evaluation of Genetic Diversity among Eight Tetraploid Wheat Varieties Using Random Amplified Polymorphic DNA (RAPD) Markers

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Abstract:

The Random amplified polymorphic DNA (RAPD) markers are used to determine the genetic diversity among eight varieties of tetraploid wheat's (Um-rabia 3, Leeds, Azeghar 1, Acsad65, Buhoth 7, Doma 1, Korfela, Um-rabia 5). The total number of the amplified bands (141 bands) are obtained, 101 of which were polymorphic, the DNA fragment size are ranged between 150 - 1650 bp, the polymorphism of RAPD markers are high (70.18%) and adequate to discriminate each variety.

The similarity matrix among wheat varieties for different similarity matrices coefficients (Nei&Li , Jaccard's, and Simple Matching (SM)) are found, the similarity value ranged between 36%-83% . The comparison among similarity coefficients using Mantel test shows that the correlation between Nei & Li and Jaccard similarity matrices was highly significant 0.99. Also, the dendrogram that constructed among wheat varieties using (UPGMA) analysis gives the lowest genetic distance between Azeghar 1 and Doma 1 varieties and the highest between Leeds and Acsad65 for both Nei & Li and Jaccard analysis , while for SM analysis the highest genetic distance was found between Um-rabia 3 and Azeghar 1 and the lowest distance was between Azeghar 1 and Doma1.

The result obtained from the current study reveals that the analysis of RAPD markers using Nei&Li and Jaccard similarity matrices are more informative than SM similarity coefficients to determined the genetic relationships among varieties and confirmed the potential of using RAPD markers to estimate the genetic diversity among wheat's varieties.

Cited from PH. D. Thesis of the first researcher.

تقييم التغاير الوراثي لثمانيه أصناف من الحنطة الرباعية باستعمال مؤشرات التضاعف العشوائي المتعدد الأشكال للحامض النووي الرايبي منقوص الأوكسجين

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ملخص البحث:

استخدمت مؤشرات التضاعف العشوائي المتعدد الاشكال لتعيين البعد الوراثي بين ثمانية اصناف من الحنطة الرباعية، (Um-rabia 3, Leeds, Azeghar 1, Acsad65, Buhoth 7, Doma 1, Korfela, Um-rabia 5) بلغ العدد الكلي للحزم المتضاعفة التي تم الحصول عليها141 حزمة.منها 101 حزمة متباينة اذ تراوح حجم الحزم من 150–1650زوج قاعدة، كان معدل نسبه التباين بين الاصناف باستخدام مؤشرات التضاعف العشوائي المتعدد الاشكال 70,18% وهذه النسبة كانت كافيه لتميز كل صنف. تم ايجاد قيم التشابه بين اصنـــاف الحنطة استنادا الى معاملات التشابه ((Nei&Li , Jaccard's, and Simple Matching (SM) ، ولقد تراوحت قيم التشابه الوراثي بين 36%–83%.

أظهرت المقارنة بين معاملات التشابه باستخدام اختبارMantel ارتباط عالي المعنوية بقيمة 0.99 بين معاملات التشابه Nei&Li , Jaccard's . بين التحليل العنقودي الذي تم تشكيله بين اصناف الحنطة باستخدام طريقة المجموعات الزوجية غير المزانة UPGMA، بأن اقل قيمة للبعد الوراثي كانت بين الصنفين Doma1 وAzeghar1 واعلى قيمة اظهرتها الشجرة العنقودية كانت بين Leeds وAcsade65 وذلك باستخدام معاملات التشابه Nei&Li , Jaccard's بينما لمعامل التشابه SM اظهرت الشجرة العنقودية اعلى بعد وراثي بين Um-rabia3 و Azeghar1 واقل قيمة كانت بين Doma1 و Azeghar1 .

أظهرت النتائج التي تم الحصول عليها من الدراسة الحالية بان تحليل مؤشرات الـــ RAPD باستخدام طريقة Nei&Li , Jaccard's كانت اكثر تماثلا مقارنة مع معامل التشابه SM لحساب العلاقة الوراثية بين الأصناف وكذلك أكدت الدراسة إمكانية استخدام مؤشرات الـــ RAPD لتقدير التنوع الوراثي بين أصناف الحنطة.

مستل من أطروحة الدكتوراه للباحث الأول

Introduction:

Wheat is one of the most vital cereal crop of the world, which constitutes a very important source of food to an enormous population. It is the most widely grown and consumed food crop of the world cultivated on a larger area and produce more tonnage of food than any other cereal[1].

Recently, the breeding programs played a great role in replacing landraces by highly-yield of genetically enhanced wheat varieties to resist the starvation worldwide and maintain food security[2]. The knowledge of diversity patterns allows plant breeders to better understand the evolutionary relationships among accessions, to sample germplasm in a more systematic fashion, and to develop strategies to incorporate useful diversity in their breeding programs[3]. The information about genetic diversity and relatedness in the available germplasm and among best breeding material is a fundamental element in plant breeding. The future of our breeding program depends upon the availability of genetic variability to increase productivity[4].

However, the study of genetic diversity based on molecular markers occupied great role in breeding programs in addition to the chemical, morphological and cytogenetic studies. Among the different types of molecular markers available is the analysis of RAPD based on the amplification of the DNA fragments with the polymerase chain reaction (PCR) starting from primers with arbitrary sequences, seem to be the reliable tools for generating useful information on polymorphism, genetic relatedness and diversity[5]. The PCR- RAPD markers are dominant markers and are extensively used in genetic mapping[6] and for the identification of markers linked with useful traits[7]. Due to their simplicity, versatility, modest cost, and ability to detect relatively small amounts of genetic variation they have been used to measure genetic diversity of many crop plants including cereals.[8]

The aims of this study are the evaluation of the usefulness of the RAPD technique in generating DNA markers to determine and identify the genetic distances among wheat varieties. Furthermore, the comparison of different similarity coefficients (Nei&Li, Jaccard and SM) in cultivated wheat were compared.

Materials and Methods

Plant materials

Eight varieties of tetraploid wheat (Um-rabia3, Leeds, Azeghar1,Aksad65, Buhoth7, Doma1, Korfela and Um-rabia5) are used in the present study. The seeds of parental varieties planted at the botanic experimental station, in the college of Agricultural and Forestry, university of Mosul, during the winter growing season of (2010-2011 and 2011-2012).

DNA Isolation

The DNA samples were extracted from wheat seeds (1g) by the CTAB (cethyltrimethylammonium bromide) method followed by an RNase-A treatment (Promega com.) for 30 min at 37^{o}C. [9]

The DNA quality was tested using 1% agarose gel electrophoresis and the quantity of extracted DNA was measured by UV-VIS spectrophotometer (UV-1800 shimadzu) at 260—280nm. The concentration of DNA was calculated according to the following formula:

DNA concentration (µg/µL) = [OD260 x 100 (dilution factor) x 50 µg/µL]/1000

The DNA samples are adjusted to the concentration of 50 ng/µL with TE buffer and subjected to Polymerase Chain Reaction (PCR) amplification. [10]

PCR Amplification and Data Analysis

The reactions of ***RAPD***-PCR were performed in a thin-walled 96-well thermal cycler (model: MultiGENE Optimax, Labnet, USA) according to Williams *et al.* (1990) with 10-mer oligonucleotides (Table1) synthesized by Biooner[5]. The final volume of 20µl contained 5µl of PCR premix from Bioneer Accu Power, [each tube in PCR premix contain: 1U DNA polymerase , 250 µ m dNTP's (dAtp,dCtp,dGtp, dTtp), 10 mM tris-Hcl(pH 9), 30mM Kcl, 1.5mM $Mgcl_2$], 3 µl of 10 pmol of each primer, 5 µl of 50 ng of DNA template and 7 µl of dH_2O. The reaction tubes were treated to the following temperature cycles: 94^{o}C for 4 minute (denaturation), followed by 36 cycles of annealing , 94^{o}C for 30 sec, 35^{o}C for 45 sec and 72^{o}C for 45 sec and a final extension of 5 min at 72^{o}C .

Table (1): Primers names and their sequences

Primer	Sequence (5´ to 3´)	Primer	Sequence (5´ to 3´)	Primer	Sequence (5´ to 3´)
BIO-RP1	CCTGGGCTTC	BIO-RP 9	CAATCGCCGT	BIO-RP 17	GTGATGGCAG
BIO-RP 2	TACGATGAAC	BIO-RP 10	GAGGATCCCT	BIO-RP 18	CAAACGTCGG
BIO-RP 3	CTTTCGTGCT	BIO-RP 11	GGCTGCAGAA	BIO-RP 19	CACACTCCAG
BIO-RP 4	GCGGTATAGT	BIO-RP 12	GGACCCCGCC	BIO-RP 20	CACAGCTGCC
BIO-RP 5	TGTACGTGAC	BIO-RP 13	ACCAGGTTGG	BIO-RP 21	GAAACGGGTG
BIO-RP 6	CTCGGGTGGG	BIO-RP 14	CTGACCAGCC	BIO-RP 22	GAAACGGGTG
BIO-RP 7	ACGGGTCTTG	BIO-RP 15	GTATTGCCCT	BIO-RP 23	GGTGCGGGAA
BIO-RP 8	CCGAATTCCC	BIO-RP 16	GCATATTCCG	BIO-RP 24	AAGTCCGCTC

The PCR products were analyzed on 2% agarose gel at 40 volt for 3h. Then the gel were stained in 0.5 µg/mL of ethidium bromide and the DNA fragments visualized under UV transilluminator. The size of the fragments were estimated based on a DNA Ladder of 100 bp (Promega com.). [11]

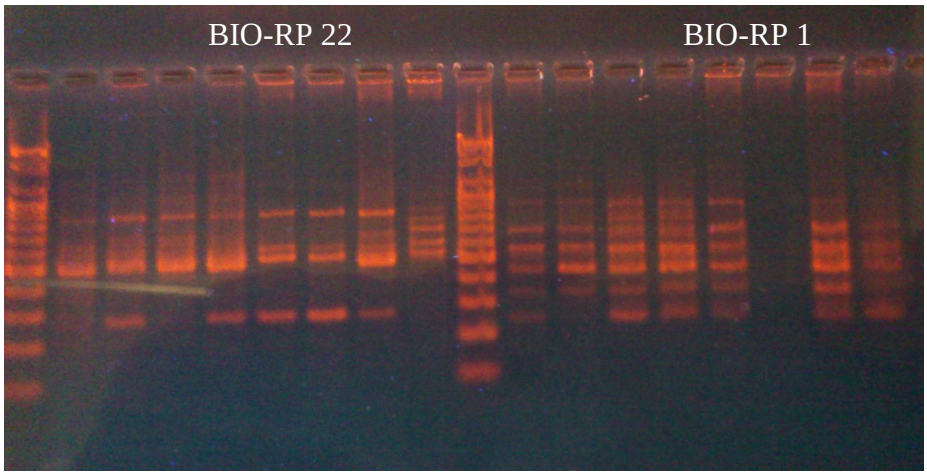


Figure (1): RAPD patterns obtained from primers BIO-RP (22, and 1) among eight wheat varieties.

The Fragments that were clearly resolved on the gels as shown in Fig.(1), were scored as 1 (present) or 0 (absent), across all the eight varieties. Bands that could not be confidently scored were regarded as missing data. The scored data obtained were analyzed to construct similarity matrices using Nei & Li,[12] Jaccared [13] and simple matching [14] similarity coefficient, the comparison among Matrix coefficients were obtained according to Mantel test [15]. The dendrogram were also constructed by un-weighted pair group method with arithmetic average (UPGMA) cluster analysis using the software NTSYS-pc: numerical taxonomy system. ver. 2.21c. (Applied Biostatics Inc.). [16]

Results and Discussion

The analysis of PCR product on 2% of agarose for eight genotype showing different degrees of polymorphism for different decamer primers (Table2). The total bands obtained are 141 bands, where 101 of which are polymorphic. These bands were produced by 22 primer from 24 decamer primer, where 2 primers generate monomorphic bands. The higher polymorphism 100% are produced by BIO-RP1, BIO-RP3, BIO-RP10, BIO-RP12, BIO-RP22, BIO-RP24 primers, whilst BIO-RP19 produced lowest polymorphism 33.33%.

The level of polymorphyism which is obtained was 70.18 % and its higher than that in some literatures, Such as Nawroz [11], who found 37% polymorphyism for tetraploid wheat and 40% for hexaploid wheat. Also, the polymorphism of durum wheat was 70% as obtained by Abd El-Haleem and *etal.* [17]

Table (2):Primers names with total bands, number of polymorphic bands and percent of polymorphyism per primer of polymorphic RAPD primers used for wheat's.

Primer	No. of fragments	No. of polymorphic bands	Degrees of polymorphism (%)	Primer	No. of fragments	No. of polymorphic bands	Degrees of polymorphism (%)
BIO-RP1	7	7	100	BIO-RP 13	10	6	60
BIO-RP 2	5	3	60	BIO-RP 14	7	4	57.14
BIO-RP 3	12	12	100	BIO-RP 15	6	4	66.66
BIO-RP 4	7	4	57.14	BIO-RP 16	11	7	63.63
BIO-RP 5	4	2	50	BIO-RP 17	5	1	20
BIO-RP 6	4	3	75	BIO-RP 18	8	6	75
BIO-RP 7	7	6	85.71	BIO-RP 19	9	3	33.33
BIO-RP 8	2	1	50	BIO-RP 20	----	Mono	----
BIO-RP 9	2	1	50	BIO-RP 21	----	Mono	-----
BIO-RP 10	3	3	100	BIO-RP 22	7	7	100
BIO-RP 11	6	5	83.33	BIO-RP 23	7	4	57.14
BIO-RP 12	7	7	100	BIO-RP 24	5	5	100
				SUM	**141**	**101**	**1544.1125**
				Average			**70.18**

The genetic similarity matrices among wheat genotype were constructed from scored data obtained using Nei & Lei , Jaccared and simple matching similarity coefficients. The obtained data shows a higher similarity value between Leeds, Acsad65 whereas low similarity value was found between Azeghar 1, Doma 1 for Nei & Li and Jaccard similarity coefficient, whilst a higher similarity value of the SM coefficient between Um-rabia 3 and Azeghar 1 are obtained and a lowest value was between Azeghar 1, Doma 1 .

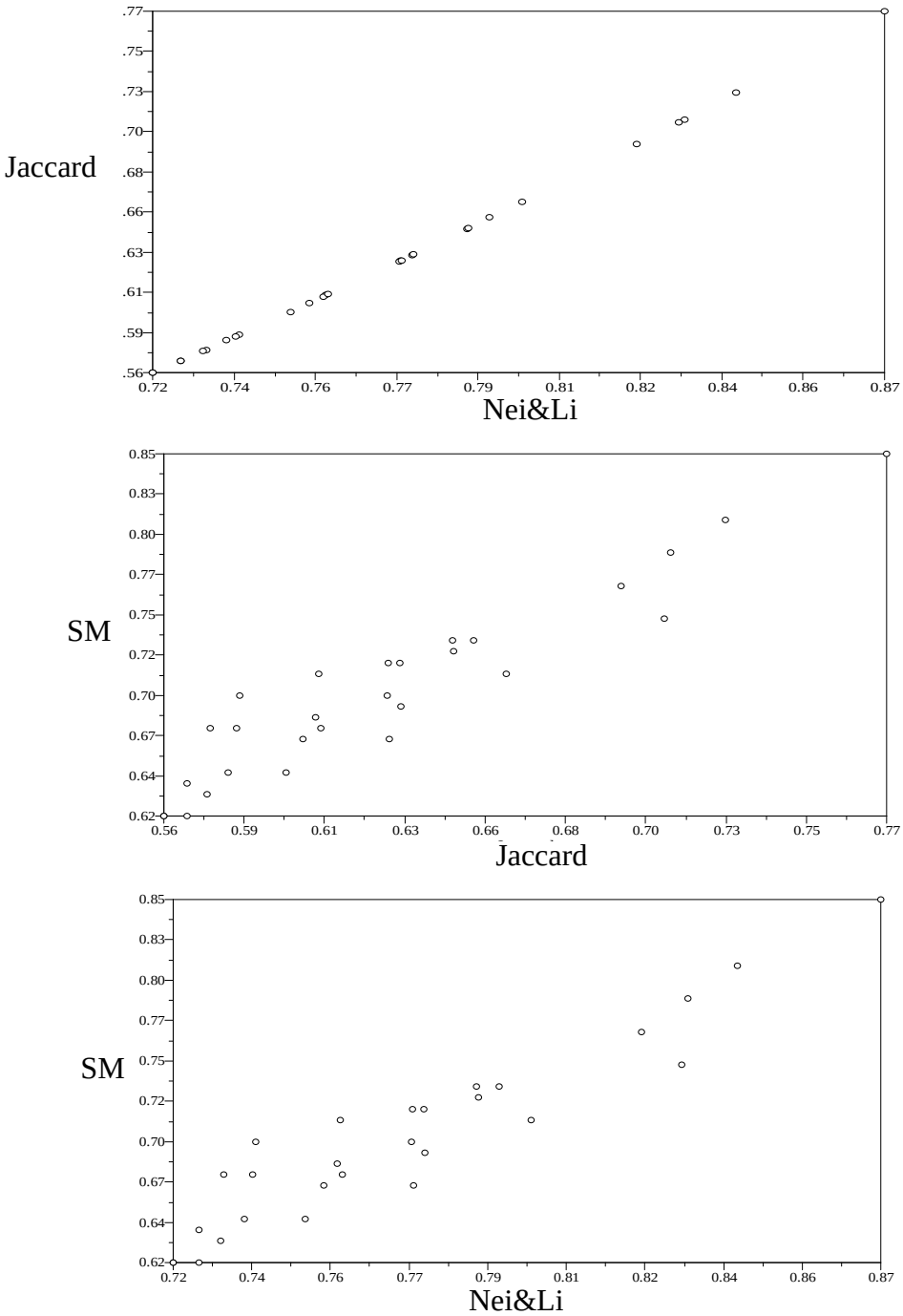
The comparison of genetic similarity coefficients (Jaccard, Nei & Li , Simple Matching) were produced according to Mantel method (Table 3),the data showed that the correlation between Nei & Li ,Jaccard similarity matrices was highly significant 0.99 whereas among SM and Nei & Li given lowest correlation 0.945. The data obtained are in agreement with the Sesli and Rabie [18,19]

Table (3) : correlation coefficients from mantel test of orginal matrices

	Nei & Li	Jaccared	SM
Nei & Li			
Jaccared	0.99932[1]		
SM	0.94563	0.94609	

[1]:Significant ($p < 0.05$)

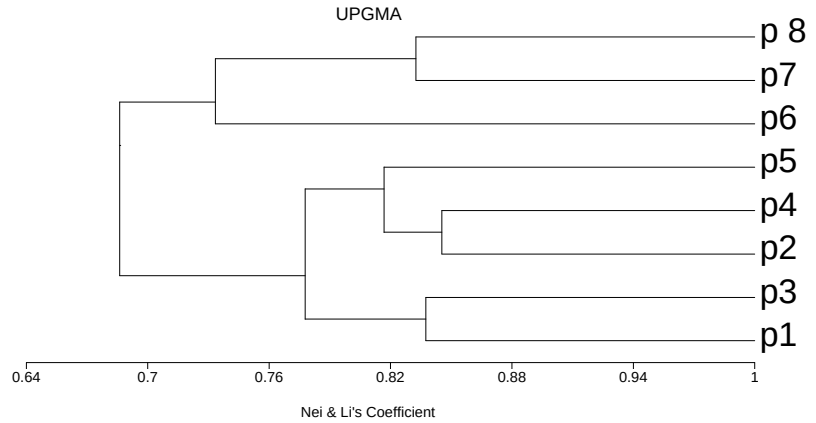
The charts of correspondence analysis between similarity coefficients in two dimensions, illustrated in Fig. (2), showing a linear relationship for Nei&Li and Jacarred with respect to the similarity matrices of SM with Nei&Li and Jaccared.



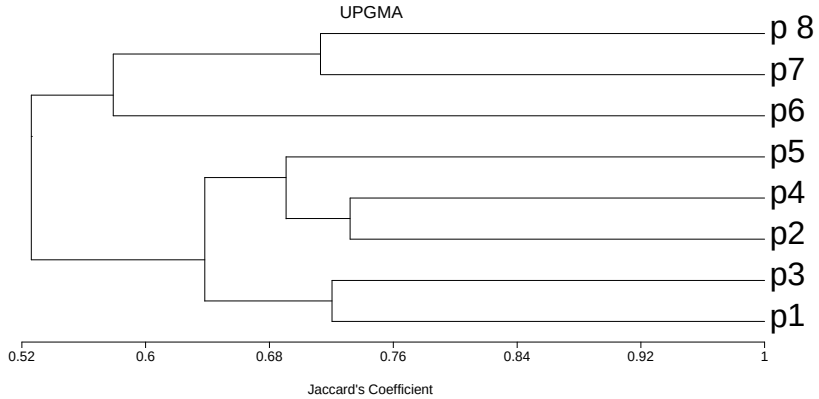
Figure(2): The relationship among Similarity matrices of Nei&Li, Jaccared and SM for tetraploid wheat based on RAPD data

Moreover, the results of the dendrogram which constructed by (UPGMA) cluster analysis for Nei&Li, Jaccard and SM are shown in Fig. (3). The data showed that the study of genetic distance among wheat genotype could be divided into two main clusters from the same node. For Nei&Li and Jaccard phenograms, the first cluster contain five varieties Um-rabia3,

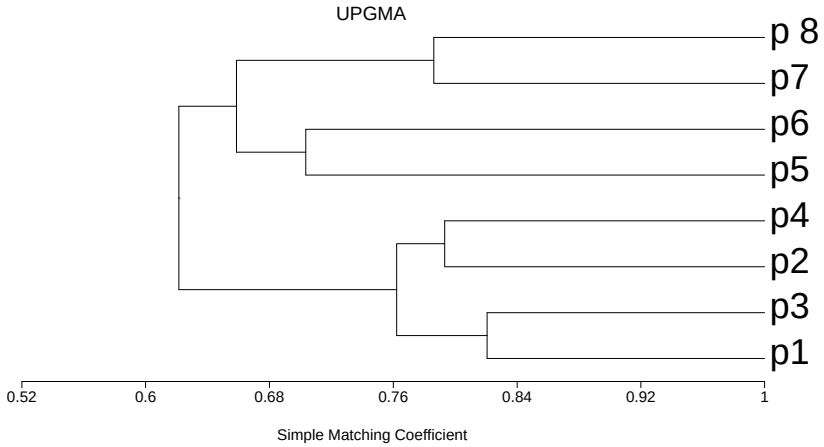
Azeghar1, Leeds, Acsad, Buhoth7 and the second cluster contain Doma1, Korfela, Um-rabia5 cultivated wheat's. But for the SM, the obtained data revealed that the first cluster contain four genotype Um-rabia3, Leeds, Azeghar1, Acsad and the second cluster include Buhouth7, Doma1, Korfela,Um-rabia5.



(a) Nei & Li



(b) Jaccared



(c) Simple Matching

Figure (3):The dendrogarms resulting from the UPGMA cluster analysis for (a) Nei & Li, (b) Jaccared (c) Simple Matching, for tetraploid wheat based on RAPD data, (P1=Um-rabia3, P2=Leeds,P3=Azeghar1, P4=Acsad65, P5=Buhoth 7, P6=Doma 1, P7=Korfela, P8=Um-rabia 5).

The charts in Fig. (3) reveals the highest genetic distance between Azeghar 1 and Doma 1 wheat varieties and the lowest distance between Leeds and Acsad65 for both Nei & Li and Jaccard analysis , while for SM analysis the lowest genetic distance was found with Um-rabia3 and Azeghar1 and the highest distance was between Azeghar 1 and Doma1.

The high genetic distance among the varieties could be attributed to the difference of the centers which developed these varieties or to the geographical locations they were grown as well as plant breeding made to it. Also, the lowest degree of genetic differences may be accredited to the usage of similar parents for constructing the varieties. Furthermore, the comparative of both charts Fig. (2, 3) , shows that the combined data set for Nei & Li and Jaccared are most closely to each other among the varieties compared to that for SM. the data obtained are in agreement with many of the studies on different wheat varieties.[20-25].

Finally, the obtained results demonstrated that the analysis of RAPD technique using Nei & Li and Jaccard similarity matrices are more informative than SM similarity coefficients to determine the genetic relationships among varieties. Also, the data confirmed the potential of using RAPD markers to estimate the genetic diversity among wheat varieties and can be helpful in our wheat breeding program.

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