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Molecular Diversity and Genetic Structure of Durum Wheat Landraces

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Abstract

To determine the genetic diversity of durum wheat, 41 accessions from Morocco, Ethiopia, Turkey, Lebanon, Kazakhstan, China, and Mongolia were analyzed through Inter-Simple Sequence Repeats (ISSR) molecular markers. Out of the used twenty primers, 15 primers that included a considerable polymorphism were selected for the analyses. Among the genotypes under study, 163 fragments (73.7%) were polymorph. Several indexes were used to determine the most appropriate primers. While UBC812, UBC864, UBC840, and UBC808 primers were among those markers which produced the highest number of bands and polymorphic bands, they also dedicated the highest rate of polymorphic index content (PIC). These primers also possessed the highest amounts of effective multiplex ratio (EMR) and marker index (MI). Therefore, these primers can be recommended for genetic evaluation of the durum wheat. The results of cluster analysis and principle component analysis indicated that the observed genetic diversity in wheat materials under study is geographically structured. The results also indicated that the genetic diversity index based on ISSR markers was higher for Turkey, Lebanon, Morocco, and Ethiopia accessions than for other countries. The high level of polymorphism in this collections durum wheat would agree with the suggestion that Fertile Crescent and parts of Africa are first possible diversity center of this crop.

Keywords: Durum wheat, Genetic diversity, Fertile Crescent, Molecular markers.

1. Introduction

Modern wheat cultivars usually refer to two species: hexaploid bread wheat, *Triticum aestivum* and tetraploid, hard or durum-type wheat, *T. durum* [21]. Durum wheat is traditionally grown around the Mediterranean Sea and it is the most common cultivated form of allotetraploid wheat. Currently, more than half of the durum wheat is still grown in the Mediterranean basin, mainly in Italy, Spain, France, Greece, West Asian, and North African countries [14, 24].

The availability of information on the genetic variation within samples and the differentiation between samples plays a significant role in the formulation of appropriate management strategies for conservation of genetic resources [15, 30]. In general, genetic diversity among and within plant species is in danger of being reduced. In wild species genetic diversity may be lost because of severe reduction in population size, whereas in domesticated crops genetic diversity may be lost because of the narrow genetic base in many breeding programmes [5]. Estimates of genetic diversity can be based on different types of data.

Molecular markers are powerful tools in study of genetic diversity, genotype description and genetic structure of wheat populations [1, 8, 12, 29].They have been widely used in genetic analyses, breeding studies and assessments of genetic diversity and relationships between cultivated species and their wild relatives, because they have numerous advantages as compared to morphological markers, including high polymorphism and independence on effects related to environmental conditions and the physiological stage of the plant [4].

Our objectives in the present study were: 1) to determine the genetic diversity in durum wheat genotypes using ISSR markers, and 2) to assess the suitability of the ISSR markers for detecting molecular variation.

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2. Materials and methods

Plant materials

The materials used in this study included 41 durum wheat landraces, all of which had been provided by ICARDA (Table 1).

ISSR analysis

DNA was extracted from leaves of young plant grown in MS culture medium [16] for about three weeks at 20 ˚C and photoperiod of 13 hours. Total cellular DNA was extracted from 0.4 g of material using the protocol described by Dellaporta *et al*. [7]. Final Pellets were solubilized in 250 µl TE solutions and kept at -20 ˚C. The quality of DNA was checked by running 5µl DNA on 0.8% agarose gel prepared in 0.5X TBE buffer. The DNA samples giving smear in the gel were rejected and extracted again. A total of 20 ISSR primers were used for PCR amplification. The PCR reaction was performed in a 25 µl volume containing the reaction buffer (10 mMTris–HCl pH 8.0, 50 mMKCl, 1.5 mM $MgCl_2$) 2 mM MgCl2, 200 µM of each dNTP, 0.2 µl primer (10 mM), approximately 35 ng of template DNA, and 2 units of Tag DNA polymerase. The assay also incorporated a sample without genomic DNA, as a negative control to rule out the possibility for self amplification of the primers or the contamination of genomic DNA. The amplification was carried out on a PalmCycler (Corbett Research Inc., Australia), with an initial 3 min denaturation at 94˚C, followed by 35 cycles of 30 s at 94˚C, 30 s at 50˚C and 1 min at 72˚C, and a final extension step for 10 min at 72˚C.Amplification products were separated on 2% agarose gel. Gels were stained with ethidium bromide and visualized with a UV transilluminator. Fifteen out of 20 ISSR primers produced high resolution bands for all samples and were used for data analyses (Table 1).

Table 1. Details on the genotypes used for assessing molecular diversity

Genotypes	Collecting region	Longitude	Latitude	Genotypes	Collecting region	Longitude	Latitude
Turkey3	Van	37°45' N	43°02' E	Lebanon2	Ynouh	33°15' N	35°18' E
Ethiopia4	Bale	7°15' N	39°32' E	Morocco6	Boulemane	33°07' N	4°29' W
China1	Tibet	29°46' N	84°01' E	Kazakhstan2	Balkhash District	45°42' N	74°20' E
China2	Tibet	29°58' N	84°30' E	China6	Tibet	29°21' N	87°32' E
Morocco2	Jerada	33°48' N	2°10' W	Ethiopia2	Jimma	8°04' N	37°16' E
Turkey4	Shirnak	37°45' N	42°53' E	Kazakhstan3	Moiynkum District	45°02' N	73°55' E
Ethiopia6	Bale	6°53' N	38°59' E	Lebanon1	Touline	33°14' N	35°26' E
Kazakhstan5	Shet District	46°50' N	73°23' E	Morocco5	Sefrou	33°38' N	4°30' W
Lebanon3	Nabatieh	33°28' N	35°26' E	Mongolia6	Burd	47°08' N	104°04' E
China3	Tibet	31°04' N	85°12' E	Turkey1	Van	37°49' N	42°52' E
Ethiopia1	East Shewa	9°04' N	39°43' E	Mongolia2	Khuld	45°15' N	105°30' E
Ethiopia5	Shinile	9°31' N	41°16' E	Turkey6	Siirt	37°46' N	41°44' E
Lebanon4	Roumine	33°28' N	35°26' E	Mongolia3	Manlai	44°22' N	107°17' E
Morocco3	Taourirt	33°58' N	2°58' W	Kazakhstan4	Moiynkum District	44°35' N	72°00' E
Lebanon5	KfarMelki	33°29' N	35°29' E	Morocco4	Khenifra	32°49' N	4°54' W
China4	Tibet	30°44' N	86°30' E	Mongolia4	Zagiin us	44°35' N	106°38' E
Morocco1	Taza	33°59' N	4°07' W	Mongolia5	Dornogovi	46°16' N	108°51' E
Kazakhstan1	Shet District	46°31' N	71°59' E	China5	Tibet	28°42' N	85°18' E
Lebanon6	Rihane	33°26' N	35°33' E	Turkey2	Batman	37°43' N	41°14' E
Mongolia1	Buren	46°34' N	105°13'E	Turkey5	Diyarbakır	37°46' N	40°55' E
Ethiopia3	East Gojjam	10°18' N	38°22' E				

Data analysis

ISSR markers were scored for the presence (1) or absence (0) of amplified bands for each of 41 landraces. The ISSR binary data matrix was used to calculate the Jaccard similarity coefficient. Similarity matrix cluster analysis was used to reveal associations among accessions based on the unweighted pair group method with arithmetic averages (UPGMA) implemented using the NTSYS-pc software version 2.02 [25]. A genetic distance [19] between the

investigated durum wheat populations was calculated on the basis of Nei's genetic distance index using the POPGENE 1.1V program [31].

For each ISSR marker, total number of amplified bands, number of polymorphic bands, and percentage of polymorphic bands (PPB) were recorded. To pass judgment on ability of ISSR markers to differentiate between wheat genotypes, polymorphism information content (PIC), effective multiplex ratio (EMR) and marker index (MI) were calculated. PIC and genetic diversity index (GD) were calculated according to the formula of Anderson *et al*. [3], as PIC and GD = 1 - Σpi^2, where *pi* is the frequency of the *i*th allele of the locus in the set of 41 wheat genotypes. EMR is the product of the fraction of polymorphic bands and the number of polymorphic bands [13]. MI was determined according to Powell *et al*. [23] as the product of PIC and EMR.

3. Results and discussion

Twenty ISSR primers were initially screened for their ability to produce polymorphic patterns across the forty one wheat landraces. Fifteen primers which were repeatable and produced high resolution bands for all the genotypes were selected for evaluation of genetic diversity in the accessions (Figure 1 and Table 2).

Table 2: The primer sequences and parameters of genetic variation generated by ISSR markers

Primer	Sequence (5'-3')	TAB	NPB	PPB	PIC	EMR	MI
UBC818	(CA)8G	17	11	64.7	0.943	7.12	6.71
UBC807	(AG)8T	12	9	75	0.816	6.75	5.51
UBC808	(CA)7G	18	14	77.8	0.944	10.89	10.28
UBC840	(GA)8Y*T	19	14	73.7	0.965	10.31	9.94
UBC825	(AC)8T	11	9	81.8	0.853	7.36	6.28
UBC848	(CA)8R*G	12	8	66.7	0.759	5.33	4.04
UBC857	(AC)8YG	10	8	80	0.851	6.5	5.53
UBC864	(ATG)6	18	14	77.8	0.913	10.89	9.94
UBC855	(AC)8YT	17	13	76.5	0.949	9.94	9.43
UBC816	(CA)8T	13	8	61.5	0.863	4.92	4.24
UBC 827	(AC)8G	17	13	76.5	0.929	9.94	9.23
UBC 812	(GA)8A	17	14	82.3	0.939	11.53	10.82
UBC 834	(AG)8YT	10	7	70	0.826	4.9	4.04
UBC 844	(CT)8RC	14	10	71.4	0.902	7.14	6.44
UBC 820	(GT)8C	16	11	68.7	0.957	7.56	7.23
Total	-	221	163	-	-	-	-
Minimum	-	10	7	61.5	0.759	4.9	4.04
Maximum	-	19	14	82.3	0.965	11.53	10.82
Mean	-	14.7	10.9	73.6	0.894	8.07	7.21

*Y: C or T, R: A or T

TAB: Total amplified bands, NPB: Number of polymorphic bands, PPB: Percentage of polymorphic bands, PIC: Polymorphism information content, EMR: Effective multiplex ratio, MI: Marker index.

ISSR polymorphism

Fifteen ISSR primers amplified a total of 221 bands in the set of forty one durum wheat accessions, of which 163 bands were polymorphic. The number of bands varied from ten (UBC857 and UBC 834) to nineteen (UBC840). The percentage of polymorphic bands (PPB) ranged between 61.5 and 82.3 with an average of 73.6% (Table 2). The mean number of bands and polymorphic bands per primer were 14.7 and 10.9, respectively. Variable efficiencies of different molecular markers for detecting DNA polymorphism in wheat have been reported. Karaca and Izbirak [10] explained that the number of polymorphic bands (58.62%) detected by ISSR markers was much higher than that of RAPD (46.02%) marker. Joshi and Nguyen [9] observed 1.8 polymorphic bands per RAPD primer among 15, while SSRs with 6.2 alleles/bands were more polymorphic [22]. The number of RFLP polymorphic bands per probe/enzyme combination in 124 bread wheat cultivars was 3.3 [20]. Altintas *et al*. [2] observed 47% polymorphism among 22 bread wheat cultivars using five AFLP and three SAMPL primer pairs with an average of 20.4 polymorphic loci per primer pair. Nagaoka and Ogihara [17] detected 3.7 polymorphisms per ISSR primer, while Carvalho *et*

al. [6] reported 12.9 polymorphic bands per primer using 18 ISSR primers in 48 wheat accessions. We detected a high level of polymorphism among the wheat genotypes using ISSRs, indicating high efficiency of the marker technique to reveal genetic diversity in the case of wheat.

The ISSR primers with dinucleotide motifs (CA)n, (GA)n and (AC)n produced a high level of polymorphism (Table 1 and 2). These results are in agreement with those of Carvalho *et al*. [6] and Najaphy *et al*. [18] who reported that dinucleotide primers were more suitable for amplifying ISSRs in bread and durum wheat.

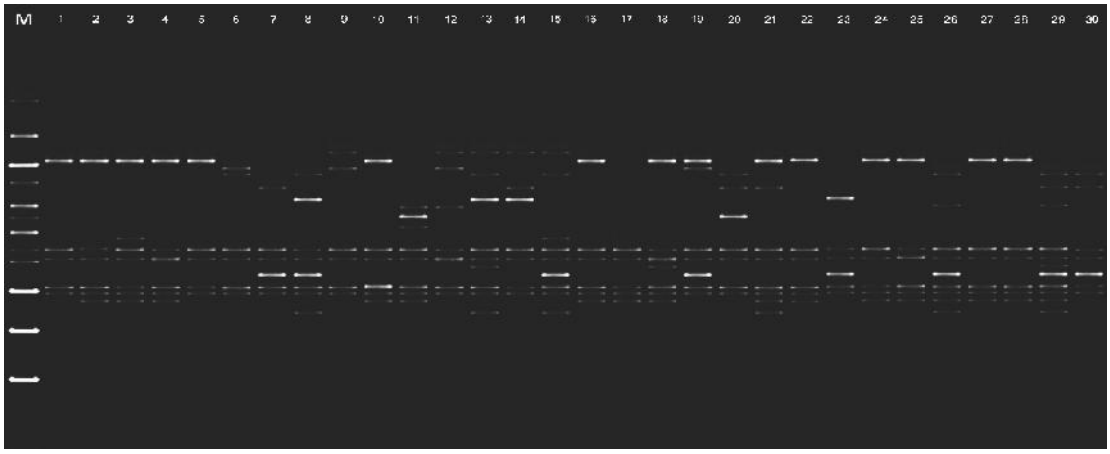

Figure 1. An example of electrophoreogram obtained with primer UBC864; the numbers indicate genotypes as listed in Table 1.

Polymorphism information content (PIC)

The PIC values for the fifteen primers varied from 0.759 to 0.965 with an average of 0.894. The lowest and highest PIC indices were recorded for primer UBC848 and UBC840, respectively. More than half of the primers showed PIC values between 0.902 and 0.965 (Table 2). The moderate values of PIC for the ISSR primers could be attributed to the diverse nature of the durum wheat accessions and/or highly informative ISSR markers used in this study.

Marker index (MI) and effective multiplex ratio (EMR)

MI is a feature of a marker and was calculated for all the primers. The MI values ranged between 4.04 and 10.82. The maximum MI was observed for the primer UBC812 and the minimum MI was obtained with ISSR primers UBC848 and UBC834. The primers that showed higher polymorphism had higher EMR values. This feature varied from 4.9 to 11.5 with a mean value of 8.07. MI was positively correlated with PIC ($r = 0.807$, $P < 0.001$) and EMR ($r = 0.993$, $P < 0.001$). A positive correlation was found between EMR and PPB ($r = 0.635$, $P < 0.05$). EMR is the product of the fraction of polymorphic bands and the number of polymorphic bands and MI is the product of PIC and EMR, therefore the higher polymorphism provides higher effective multiplex ratio and marker index. These two features have been used to evaluate the discriminatory power of molecular marker systems in some plant species e.g. apricot (ISSR, EMR = 4.8, MI = 3.74) [13], Jatropha (AFLP, EMR = 97, MI = 25.13) [26], Pongamia (AFLP, EMR = 77.2, MI = 16.83) [27].

Yet, the overall results showed that UBC812, UBC864, UBC840, and UBC808 primers have the highest number of bands, polymorphic bands, and polymorphic index content (PIC). These primers also acquired the highest amount of MI and EMR. Consequently, these ISSR primers could be considered the most appropriate markers for the genetic diversity studies in this durum wheat material. The results of present study also showed that ISSR analysis is quick and reliable. The marker system provided sufficient polymorphism and reproducible fingerprinting profiles for evaluating genetic diversity of durum wheat genotypes. Molecular variation assessed in this study in combination with agronomic and morphological characters of wheat can be exploited in breeding programmes.

Genetic relationships among durum wheat genotypes and populations

Jaccard similarity matrix based on ISSR binary data was used to group the wheat accessions using the complete linkage method. The dendrogram obtained ¨with the use of this method had in comparison with

the UPGMA method a higher cophenetic correlation and no chaining (Figure 2).

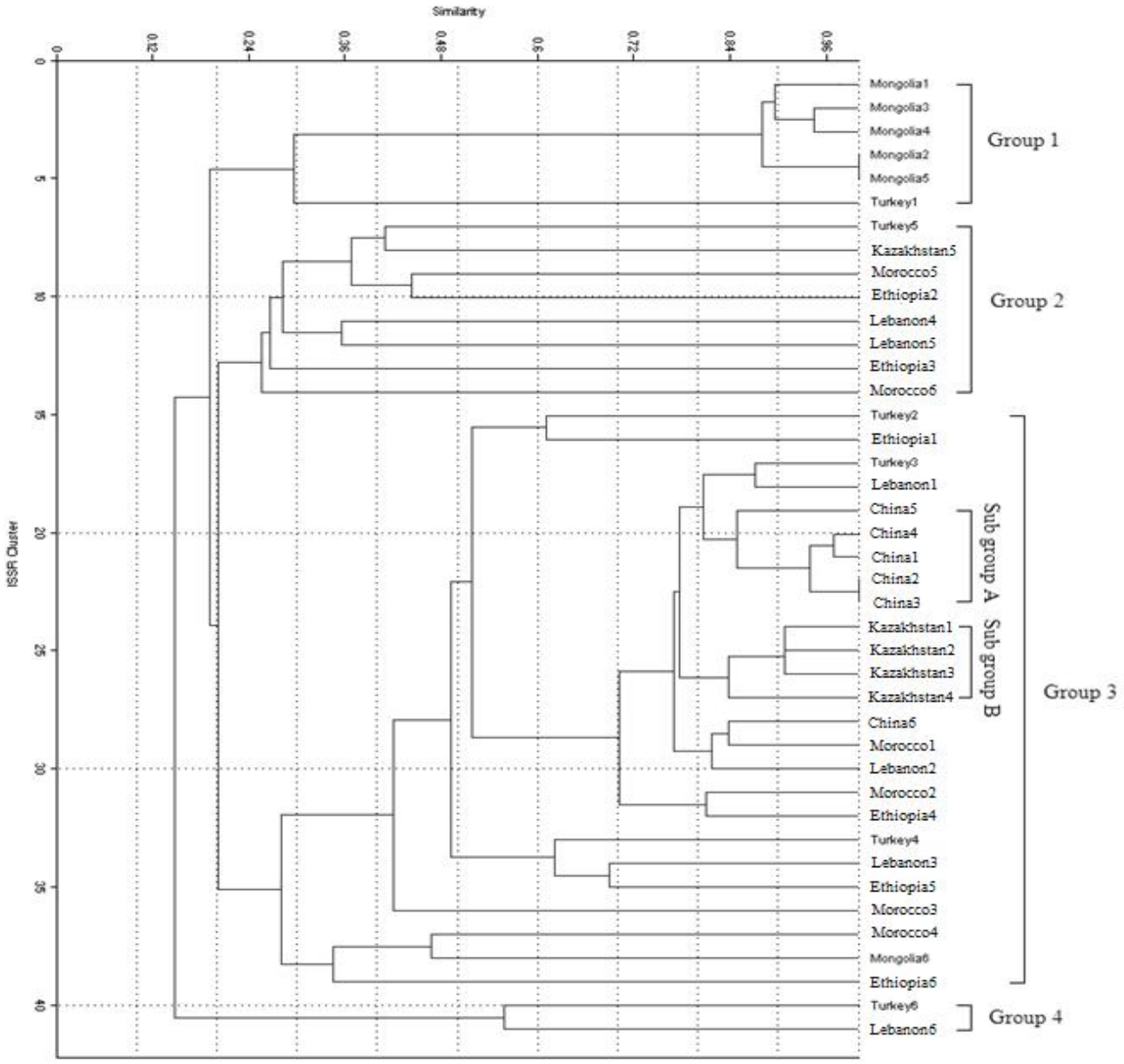

Figure 2: Dendrogram showing the relationship among 41 durum wheat genotypes, generated by UPGMA cluster tree analysis. The scale is based on Jaccard's similarity index.

Accessions studied were clustered into four distinct groups at the similarity level I = 0.24. The first group included five genotypes from Mongolia and one accession from Turkey. At the same time, one genotype from Turkey, two genotypes from Morocco along with samples from Kazakhstan, Lebanon, and Ethiopia were put in the second group. More than 83% of the durum wheat accessions from China were put in third group (subgroup A). This result points to the striking similarities between the samples in this population. Similar results were also characteristic of the Kazakhstan population samples. Four of the five genotypes in this population were put in belonged to subgroup B (group 3). Group three which contained61% of the examined samples included also genotypes 2, 3, and 4 from Turkey,genotypes1, 4, 5, and 6 from Ethiopia, genotypes 1, 2, and 3 from Lebanon,genotypes1, 2, 3, and 4 from Moroccoand accessions from China, Kazakhstan and Mongolia. Group four only included genotypes number 6 from Turkey and Lebanon. Placing these samples in a separate group indicates their genetic distance from other genotypes. No phenomenon in plant breeding has yet been as influential on increasing agricultural

products as hybrid varieties. With regard to this fact, crossing two individuals with less genetic affinity can lead to genotypes with more capabilities through heterosis. One of the main applications of these clusters is the estimation of the genetic distance between genotypes. Therefore, these results can be used in identifying appropriate parents for crossings and creating superior hybrids in hybridization.

The study of the genetic distance between populations indicated that the closest distance existed between accessions from Morocco and Turkey, with a distance rate of 0.06. Right after these populations, Ethiopian genotypes were the closest populations to Morocco and Turkey samples with the respective distance rates of 0.065 and 0.069. At the same time, Mongolian genotypes were the farthest population from Morocco and Turkey with the distance rates of 0.445 and 0.441. Overall, the farthest populations compared to other populations, was Mongolia (Figure 3 and Table 3).

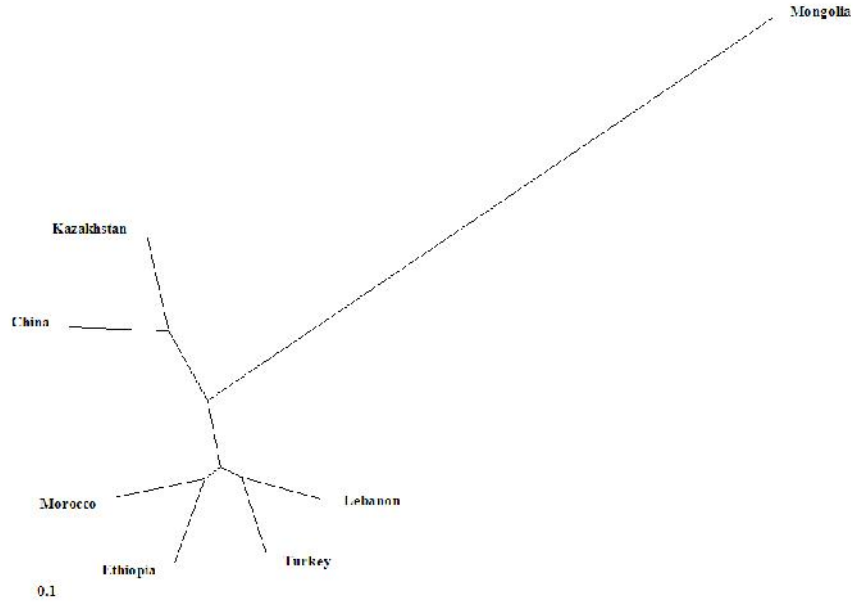

Figure 3: Dendrogram showing the genetic distance among studied durum wheat populations based on ISSR polymorphism. The distance was calculated according to Nei [19].

Table 3: Genetic distances among the studied durum wheat populations based on ISSR data.

Populations	China	Ethiopia	Kazakhstan	Lebanon	Mongolia	Morocco	Turkey
China	0.000						
Ethiopia	0.149	0.000					
Kazakhstan	0.071	0.106	0.000				
Lebanon	0.135	0.071	0.121	0.000			
Mongolia	**0.455**	0.225	**0.441**	0.258	0.000		
Morocco	0.115	**0.065**	0.093	0.081	0.282	0.000	
Turkey	0.175	**0.069**	0.122	**0.060**	0.207	0.096	0.000

Genetic diversity indexes

According to ISSR markers polymorphism, the highest genetic diversity with the rate of 0.810 and the mean pattern number of 5.67 belonged to the African (i.e. Moroccan and Ethiopian) durum wheat. In addition to these accessions, samples from Turkey and Lebanon with the genetic diversity indices of 0.806 and 0.803 and the mean pattern numbers 5.67 and 5.47 were among the most diverse genotypes. As we move from Africa and western Asia towards eastern Asia, the genetic diversity of landraces shrinks significantly (Table 4). Samples taken from Kazakhstan and Mongolia with the respective average diversity indexes of 0.549 and 0.414 and the mean pattern numbers of 3.13 and 2.53 showed less diversity compared to African and west Asian accessions. At the same time, samples from the farthest eastern area which was studied (China) showed the least variety with a diversity rate of 0.348 and the pattern number of 2.27. Considering these

evidences, one might be inclined to conclude that East Asian areas can not be considered as important centers for the diversity of durum wheat. Instead, as the results of this study show, areas in west Asia (Fertile Crescent) and north and east of Africa are considered to be the main diversity centers for this plant. Generally speaking, great genetic variation should exist in the center of origin and domestication. Vavilov [28] also reported that the Middle, Near East regions, and North Africa are considered the centers of origin and diversification of durum wheat.

Table 4: Genetic diversity and number of patterns of 41 durum wheat accessions from seven different origins based on ISSR data.

Kazakhstan		Mongolia		China		Ethiopia		Morocco		Lebanon		Turkey		
GD.	NP.	GD.	NP.	GD.	NP.	GD.	NP.	GD.	NP.	GD.	NP.	GD.	NP.	Markers
0.72	4	0.278	2	0.278	2	0.832	6	0.832	6	0.777	5	0.832	6	UBC818
0.32	2	0.278	2	0.499	3	0.666	4	0.832	6	0.832	6	0.666	4	UBC807
0.56	3	0.278	2	0.499	3	0.832	6	0.832	6	0.832	6	0.832	6	UBC808
0.8	5	0.611	3	0.722	4	0.832	6	0.832	6	0.832	6	0.832	6	UBC840
0.32	2	0.278	2	0.499	3	0.832	6	0.777	5	0.777	5	0.832	6	UBC825
0.32	2	0.499	3	0.000	1	0.777	5	0.777	5	0.777	5	0.832	6	UBC848
0.32	2	0.278	2	0.278	2	0.832	6	0.832	6	0.777	5	0.777	5	UBC857
0.72	4	0.499	3	0.278	2	0.832	6	0.832	6	0.777	5	0.832	6	UBC864
0.72	4	0.499	3	0.278	2	0.832	6	0.832	6	0.832	6	0.832	6	UBC855
0.72	4	0.611	3	0.444	2	0.777	5	0.666	4	0.777	5	0.832	6	UBC816
0.56	3	0.278	2	0.000	1	0.832	6	0.777	5	0.832	6	0.832	6	UBC827
0.56	3	0.666	4	0.278	2	0.832	6	0.832	6	0.832	6	0.832	6	UBC812
0.32	2	0.278	2	0.278	2	0.777	5	0.832	6	0.777	5	0.666	4	UBC834
0.56	3	0.278	2	0.278	2	0.832	6	0.832	6	0.777	5	0.832	6	UBC844
0.72	4	0.611	3	0.611	3	0.832	6	0.832	6	0.832	6	0.832	6	UBC820
0.549	3.13	0.414	2.53	0.348	2.27	0.810	5.67	0.810	5.67	0.803	5.47	0.806	5.67	Mean

NP: Number of patterns, GD: Genetic diversity index

4. Conclusion

The present study confirms that, areas in west Asia and north and east of Africa are considered to be the main diversity centers of durum wheat crop. ISSR analysis provided sufficient polymorphism and reproducible fingerprinting profiles for evaluating genetic diversity of durum wheat accessions. PIC, MI and EMR are proposed as marker parameters for selecting informative primers. The polymorphism detected among the studied durum wheat accessions can be used in breeding programs to maximize the use of genetic resources.

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