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Research Article

The Use of ISSR and RAPD Markers for Genetic Diversity among South Tunisian Barley

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Random amplified polymorphic DNA (RAPD) and intersimple sequence repeat (ISSR) were assayed to determine the genetic diversity of 80 barley specimens from South Tunisia. The ISSR primers showed variation in the percentage of polymorphism, band informativeness (Ib), and resolving power (Rp). The percentage of polymorphism is 66.67%, the average Ib ranged from 0.24 to 0.39, while Rp ranged from 0.74 to 1.16. In RAPD analysis, three primers yielded a total of 17 scorable bands, which are all polymorphic. The three polymorphic primers exhibited variation with regard to average band informativeness (AvIb) and resolving power (Rp). RAPD and ISSR marker systems were found to be useful for the genetic diversity among the barley specimens. The two dendrograms obtained through these markers show different clustering of 80 barely specimens, but we noted that some clusters were similar in some cases. A poor correlation ($r = 0.12$) was found between both sets of genetic similarity data, suggesting that both sets of markers revealed unrelated estimates of genetic relationships. Therefore, the ISSR and RAPD molecular markers show two genetic grouping of studied barely specimens.

1. Introduction

Barley (*Hordeum vulgare* L.) is one of the most important crop species in the World and has been subject to considerable genetic studies. It is a diploid ($2n = 2x = 14$), largely self-fertilizing species with a large genome [1].

Barley is cultivated on about 450.000 hectares in Tunisia. During centuries, early domestication and local knowledge have generated diverse local barley used mainly for feed and lowly for food [2]. In semiarid regions, barley is mostly cultivated by sheep owners and gazed one or two times as early winter crop when forage and pasture are not available. The conservation and use of plant genetic resources are essential to the continued maintenance and improvement of agricultural production.

The identification of varieties of crop plants has become increasingly important to the documentation of genetic resources and to the protection of the breeders' interests. To the malting and brewing industries, this is especially important because different varieties of barley (*Hordeum vulgare* L. ssp. *vulgare*) have widely different qualities and use characteristics. Farmers need positive identification for the protection of their proprietary rights on varieties. The grower needs assurance that the seed lot is of the variety he intends to use. Processors must be assured of varietal identity and that it is free from mixtures. Examination of grain morphological characteristics is the standard method of identifying barley cultivars, but not all of them can be distinguished on this basis. Several biochemical techniques have been used to complement morphological examination of barley cultivars, and most of them rely on variations among isoenzymes [3] and seed storage proteins [4]. Nevertheless, characterization with these kinds of markers was not very efficient for barley varieties due to the low levels of allelic variation in many isoenzymatic loci, the high degree of genetic relationship among the different varieties, and the high degree of polymorphism within barley varieties.

Molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and populations. It has been showed that different markers might reveal different classes of variation [5, 6]. It is correlated with the genome

fraction surveyed by each kind of marker, their distribution throughout the genome and the extent of the DNA target which is analyzed by each specific assay [7]. The advent of the polymerase chain reaction (PCR) favored the development of different molecular techniques such as random amplified of polymorphic DNA (RAPD), simple sequence repeats (SSR or microsatellite), sequence-tagged sites (STS), random amplified microsatellite polymorphism (RAMP), and intersimple sequence repeat polymorphic DNA (ISSR) [8]. These molecular markers had been used in barley for detecting genetic diversity, genotype identification, and genetic mapping [9–11]. Of these techniques, RAPD has several advantages, such as simplicity of use, low cost, and the use of small amount of plant material. RAPDs were proved to be useful as genetic markers in the case of self-pollinating species with a relatively low level of intraspecific polymorphism, such as hexaploid wheat [12, 13] and cultivated barley [14]. Bernard et al. [15], used RAPD markers and have revealed extensive polymorphism between different genotypes of wild barley. They showed that out of total genetic diversity estimated, 75% of the variation detected was partitioned within the genotypes and 25% among the populations, whereas no substantial differences were found between countries. ISSR markers, which involve PCR amplifications of DNA using a primer, composed of a microsatellite sequence anchored at 3′ or 5′ end by 2–4 arbitrary, could be used to assess genetic diversity [16]. ISSRs have been used for cultivar identification for potatoes [17], wheat [18], bean [19], and barley [10, 11].

In this study, we evaluate the level and organization of the genetic diversity and relationship in barely specimens cultivated in south Tunisia using RAPD and ISSR markers, in order to establish a base line to assist future conservation and breeding programmes of this species. Also we aim to report the usefulness of RAPD and ISSR for the assessment of genetic diversity and relationships among barley specimens.

2. Materials and Methods

2.1. Plant Material. Eighty barley (*Hordeum vulgare* L.) specimens selected from "Institut des régions arides de Médenine" (South Tunisia) were used in this study. The specimen numbers and country of origin are listed in Table 1.

2.2. DNA Extraction. Total DNA was extracted from fresh leaves as described by J. J. Doyle and J. L. Doyle [20] with some modifications. DNA concentration was determined by both spectrophotometry at 260 nm and by 2% agarose gel electrophoresis.

2.3. ISSR-PCR Analysis. A set of 10 ISSR primers was procured from Operon molecular for life (Table 2). Initially 3 specimens were used for PCR amplification using all the 10 primers. Three primers gave clear and polymorphic patterns and were used for further analysis of 80 specimens. For each primer, 20 μL amplification reaction contained 2.5 μL buffer (Taq Buffer avec $(NH_4)_2SO_4$ 5x), 100 ng of genomic DNA, 2 mM of $MgCl_2$ and 1 U of Taq DNA polymerase. PCR amplifications were performed in gen-Amp PCR 9700 thermal cycler system, with initial denaturation at 94°C for 5 min followed by 35 cycles: denaturation at 94°C for 1 min, annealing at 36°C for 1 min, extension at 72°C for 2 min, with final extension at 72°C for 7 min. PCR products were separated on 2% agarose gels, stained with ethidium bromide, and visualised on UV. The gel was photographed using Bio-print camera.

2.4. RAPD-PCR Analysis. RAPD analysis was carried out with 10 decamer random primers from Operon molecular for life (Table 2). PCR amplifications were carried out also with 3 specimens. Three primers gave clear and polymorphic amplification patterns and were used for further analysis of 80 specimens. For each primer, 20 μL amplification reaction contained: 100 ng of genomic DNA, 5 mM of $MgCl_2$, 1 U of Taq DNA polymerase, and 4 μL de buffer (Taq Buffer avec $(NH_4)_2SO_4$ 10x). PCR amplifications were performed in a gen-Amp PCR 9700 thermal cycler system. The PCR conditions included initial denaturation at 94°C for 5 min, followed by 45 cycles: denaturation at 92°C for 1 min, annealing at 50°C for 2 min and extension at 72°C for 2 min with final extension at 72°C for 7 min.

2.5. Reproducibility of Amplification Patterns. DNA amplifications with each ISSR and RAPD primers were repeated at least thrice to ensure reproducibility. The bands were considered reproducible and scorable only after observing and comparing them in three separate amplifications for each primer. Clear and intense bands were scored while faint bands against background smear were not considered for the further analysis.

2.6. Scoring and Data Analysis. For each specimen, each fragment/band that was amplified using ISSR and RAPD primers was treated as unit character. Molecular weight of each of the potential specific bands was calculated using the software Gel-pro analyser. Unequivocally scorable and consistently reproducible amplified DNA fragments were transformed into binary character matrices (1 for presence, 0 for absence). The commercial software package SPSS 16 was used to develop similarity matrices based on the Dice coefficient which is defined as $2a/2a + u$, where "a" is the number of positive matches and "u" is the number of nonmatches. These data were then used to construct dendrogram for cluster analysis based on the Dice coefficient and on the simple link as the algorithm aggregation method. Two separate dendrograms for ISSR and RAPD data were generated. The distance matrices obtained in RAPD and ISSR analyses were compared using correlation analysis. Average band informativeness (AvIb) is a measure of closeness of a band to be present in 50% of the genotypes under study, and resolving power (Rp) is the sum of Ib values of all the bands amplified by a primer. Band informativeness (Ib) and resolving power (Rp) were calculated as given by Prevost and

Table 1: Studied barley specimens and their geographical origins.

	Specimens name	Code	Location	Region	Provence
1	Elmejni	**19**	Elmejni	Gabes	Gabes
2	Elmdou	**43**	Elmdou	Gabes	Gabes
3	Mazreet ben slama	**74**	Mazreet ben slama	Gabes	Gabes
4	Mareth	**10**	Mareth	Mareth	Gabes
5	Aiin tounine	**69**	Aiin tounine	Matmata	Gabes
6	Dkilet toujene	**17**	Dkilet toujene	Matmata	Gabes
7	Matmatta jdida 1	**30**	Matmatta jdida	Matmata	Gabes
8	Matmata jdida 2	**34**	Matmata jdida	Matmata	Gabes
9	Zmerten	**50**	Zmerten	Matmata	Gabes
10	Belkir	**20**	Belkir	Belkir	Gafsa
11	Belkhir 3	**22**	Belkhir	Belkhir	Gafsa
12	Essaidane	**29**	Essaidane	Ben Guerdane	Medenine
13	Bniri	**48**	Bniri	Ben guerdane	Medenine
14	Oued erbaii	**49**	Oued erbaii	Ben guerdane	Medenine
15	Jellala	**70**	Jellala	Ben guerdane	Medenine
16	Oued el khil 2	**4**	Oued el khil	Ben keddache	Medenine
17	Labyar 2	**11**	Labyar	Ben keddache	Medenine
18	Manzel mgor 1	**26**	Manzel mgor	Ben khddache	Medenine
19	Manzel mgor 2	**27**	Manzel mgor	Ben khddache	Medenine
20	Labyar 1	**35**	Labyar	Ben khddache	Medenine
21	Switir 1	**77**	Switir	Medenine	Medenine
22	Swittir	**12**	Swittir	Medenine	Medenine
23	Bir ezwai	**13**	Bir ezwai	Medenine	Medenine
24	El bhira 1	**31**	El bhira	Medenine	Medenine
25	El bhira 2	**32**	El bhira	Medenine	Medenine
26	Hjar	**36**	Hjar	Medenine	Medenine
27	Tarf ellil	**41**	Tarf ellil	Medenine	Medenine
28	Ben khddache centre	**44**	Ben khddache centre	Medenine	Medenine
29	Bir ezzwai	**46**	Bir ezzwai	Medenine	Medenine
30	Ferjania 2	**53**	Ferjania	Medenine	Medenine
31	Oued elhalouf	**56**	Oued elhalouf	Medenine	Medenine
32	Ksar ejdid	**59**	Ksar ejdid	Medenine	Medenine
33	Lagrabette	**63**	Lagrabette	Medenine	Medenine
34	Ben gzayel	**73**	Ben gzayel	Medenine	Medenine
35	Thahret el gbour 2	**57**	Thahret el gbour	Medenine	Medenine
36	Thahret elgbour	**71**	Thahret elgbour	Medenine	Medenine
37	Errssifett	**37**	Errssifett	Zarzis	Medenine
38	Essolb	**51**	Essolb	Zarzis	Medenine
39	Orge Pakestani	**79**	Orge Pakestani	Pakistan	Pakistan
40	Echahbania 1	**2**	Echahbania	Tataouine	Tataouine
41	Tataouine ejdida	**3**	Tataouine ejdida	Tataouine	Tataouine
42	El bagbag 3	**6**	El bagbag	Tataouine	Tataouine
43	Lamaat	**7**	Lamaat	Tataouine	Tataouine
44	El ferch 1	**8**	El ferch	Tataouine	Tataouine
45	Ksar ouled boubaker	**9**	Ksar ouled boubaker	Tataouine	Tataouine
46	Tlalite	**14**	Tlalite	Tataouine	Tataouine
47	Bir 30	**15**	Bir 30	Tataouine	Tataouine
48	Oued el khil	**16**	Oued el khil	Tataouine	Tataouine
49	Amadi	**18**	Amadi	Tataouine	Tataouine
50	Mgitt 2	**21**	Mgitt	Tataouine	Tataouine

Table 1: Continued.

	Specimens name	Code	Location	Region	Provence
51	Missawa	**23**	Missawa	Tataouine	Tataouine
52	Gomrassen	**24**	Gomrassen	Tataouine	Tataouine
53	Gattouffa	**25**	Gattouffa	Tataouine	Tataouine
54	Bir lahmer 2	**28**	Bir lahmer	Tataouine	Tataouine
55	El bag bag 1	**33**	El bag bag	Tataouine	Tataouine
56	Erremtha 2	**38**	Erremtha	Tataouine	Tataouine
57	El ferch 2	**39**	El ferch	Tataouine	Tataouine
58	Oued el khil 3	**42**	Oued el khil	Tataouine	Tataouine
59	Misstawa1	**45**	Missawa	Tataouine	Tataouine
60	Grager 2	**47**	Grager	Tataouine	Tataouine
61	Gormassa	**52**	Gormassa	Tataouine	Tataouine
62	Ksar oun 1	**54**	Ksar oun	Tataouine	Tataouine
63	Bir addim	**55**	Bir addim	Tataouine	Tataouine
64	Ksar ouled dbab	**58**	Ksar ouled dbab	Tataouine	Tataouine
65	El mawouna	**60**	El mawouna	Tataouine	Tataouine
66	Ezzahra 2	**61**	Ezzahra	Tataouine	Tataouine
67	Elmziraa	**62**	Elmziraa	Tataouine	Tataouine
68	Bouzrida	**64**	Bouzrida	Tataouine	Tataouine
69	Echahbania	**65**	Echahbania	Tataouine	Tataouine
70	Gormassa 2	**66**	Gormassa	Tataouine	Tataouine
71	Lahyet mars	**67**	Lahyet mars	Tataouine	Tataouine
72	Gomrassen 1	**68**	Gomrassen	Tataouine	Tataouine
73	Elbagbag 2	**72**	Elbagbag	Tataouine	Tataouine
74	Gragre 1	**75**	Gragre	Tataouine	Tataouine
75	Oued el khil 1	**76**	Oued el khil	Tataouine	Tataouine
76	Chenenni	**78**	Chenenni	Tataouine	Tataouine
77	Gasbett gomri	**5**	Gasbett gomri	Tataouine	Tataouine
78	Chehbania 2	**40**	Chehbania	Tataouine	Tataouine
79	El mourra	**1**	El mourra	Tataouine	Tataouine
80	Rihane	**80**	Rihane	Tunis	Tunis

Wilkinson [17]. The formulae used for the above-mentioned parameters are

(i) Band informativeness of a given band: $\mathrm{Ib} = 1 - (2 \times |0.5 - p|)$, where p is the proportion of the total genotypes containing the band;

(ii) resolving power of a primer is the sum of band informativeness: $\mathrm{Rp} = \Sigma \mathrm{Ib}$.

The hierarchical classification ascendant (HCA) was conducted on ISSR and RAPD data based on dissimilarity (Dice index) and the simple link as the algorithm aggregation method.

3. Results

3.1. Identification and Evaluation of RAPD and ISSR Primers for Diversity Estimation. Out of 10 decamer random primers used for initial screening with three representative genotypes, only three primers amplified polymorphic patterns. These primers were then used for RAPD analysis of all the 80 genotypes. Amplification products of the 80 genotypes with these three primers yielded a total of 17 scorable bands, which are all polymorphic (Table 3). The highest number of bands (8) was obtained with primer BY-15, while the lowest number (4) was obtained with primer UBC-402. Different primers showed the same variation in their ability to detect polymorphism (100%). The three polymorphic primers exhibited variation with regard to average band informativeness (AvIb) and resolving power (Rp). The AvIb and Rp values of these polymorphic primers have been depicted in Table 6. The primer OPA-11 showed the lowest AvIb (0.37) and Rp (1.85), while the highest AvIb (0.65) and Rp (5.20) values were exhibited by the primers BY-15.

For ISSR markers, a total of 10 primers consisting of di- and tri-repeat motifs were used for initial screening with 3 specimens. Out of these, 7 primers gave no amplification at all, while only 3 primers were found to give clear and polymorphic patterns and were subsequently used to analyze the entire set of 80 genotypes. These ISSR primers amplified a total of 9 bands out of which 6 bands were polymorphic. These primers showed variation in the percentage of polymorphism band informativeness (Ib) and resolving power (Rp). The percentage of polymorphism is 66.66%;

Table 2: ISSR and RAPD primers tested in this study.

	ISSR primers	Sequence of primer (5′–3′)
1	UBC-888	BDBCACACACACACACA
2	UBC-890	VHVGTGTGTGTGTGTGT
3	A12	$(GA)_6CC$
4	UBC-810	GAGAGAGAGAGAGAGAT
5	UBC-812	GAGAGAGAGAGAGAGAA
6	UBC-814	CTCTCTCTCTCTCTCTA
7	UBC-815	CTCTCTCTCTCTCTCTG
8	UBC-822	TCTCTCTCTCTCTCTCA
9	UBC-834	AGAGAGAGAGAGAGAGYT
10	UBC-845	CTCTCTCTCTCTCTCTRG
	RAPD Primers	Sequence of primer (5′–3′)
1	UBC- 402	-CCCGCCGTTG-
2	UBC-475	-CCAGCGTATT-
3	UBC-490	-AGTCGACCTT-
4	UBC-534	-CACCCCCTGC-
5	UBC-102	-GGTGGGGACT-
6	OPA-04	-AATCGGGCTG-
7	OPA-18	-AGGTGACCGT-
8	OPA-11	-CAATCGCCGT-
9	BY-15	-CTCACCGTCC-
10	W07	-CTGGACGTCA-

the average Ib ranged from 0.24 to 0.39 while Rp ranged from 0.74 to 1.16 (Table 3). The primer UBC 890 showed the highest values of average Ib (0.39) and Rp (1.16).

3.2. Genetic Diversity and Clustering Based on RAPD and ISSR Polymorphism Data. The dendrogram obtained using RAPD data indicates nine main clusters (Figure 2, Table 5). The cluster 8 includes the specimen "Lahyet mars" collected from Tataouine, characterized by the low number of locus (only 3). The class 7 includes only two specimens "Thahet el gbour 2" from Médenine and "Grager 2" from Tataouine, which present the height number of locus (ten loci). The cluster 1 includes the majority of specimens (54 specimens), characterized by the absence of 2 locus having molecular size 900 and 390 pb, respectively. The position of specimens regrouped in clusters 6, 9, 11, 12, 13, 14, and 15 obtained by ISSR markers (Table 4), remained the same as in the RAPD dendrogram clusters 2, 4, 5, 6, 7, 8, and 9.

According the ISSR data, a dendrogram was developed for 80 genotypes and indicates 18 main clusters; the cluster 5 includes 29 specimens (Table 3), characterized by the presence of 7 locus with molecular size ranging from 225 to 400 bp. The cluster 4 includes 18 specimens, characterized by the presence of 6 loci whose molecular size ranged from 225 to 300 bp. The different clusters 1, 7, 12, and 18 would constitute one group characterized by the presence of 6 loci with variable molecular size. The cluster 13 includes two specimens "Ezzahra 2" and "Bouzrida" collected from Tataouine, having low number of bands: 2 loci whose molecular sizes were 275 and 295 pb. The specimen "Bir addim" from Tataouine included in cluster 11, covering 3 loci with molecular sizes 225, 275, and 300 pb.

3.3. Comparison of RAPD and ISSR Markers in Diversity Assessment of Barley Genotypes. The composition of clusters obtained using independently RAPD and ISSR markers have revealed similar groupings in only some clusters. The performance of these markers was evaluated using various parameters such as percentage of polymorphism, average band informativeness, resolving power, and clusters formed in the dendrogram. The comparison of these parameters done using two marker systems is summarized in Table 6. Percentage of polymorphic markers: the three ISSR primers yielded average three bands per primer, while the three RAPD primers amplified average 5.66 bands per primer. The average number of polymorphic bands per primer was higher in case of RAPDs (5.22) as compared to that in ISSRs (2). The range of band informativeness (Ib) values of both marker systems is reported in Table 6. The highest value (0.49) displayed by RAPD markers is higher than the ISSR markers (0.30). Resolving power is a characteristic of a primer which reflects overall suitability of a marker system for the purpose of identification, as it is related to the number of specimens distinguished by that primer [17]. Rp value for both RAPD and ISSR polymorphic primers was calculated, and it was observed that RAPD primers had greater Rp (2.99) than ISSR primers (0. 92).

The correlation coefficient for the elements of the RAPD GS (Genetic Similarity) and ISSR-GS matrices was calculated using the mantel test [21]. There was no significant correlation ($r = 0.12$) between the RAPD GS and ISSR-GS matrices, indicating that both sets of markers revealed the unrelated estimates of genetic relationships.

4. Discussion

The results indicated that the percentage of RAPD polymorphic bands (100%) was higher than that of ISSR (66.67%). The mean number of amplification RAPD bands (5.66) was more than that of ISSR (3). Moreover, the total number of polymorphic bands (17) detected by three RAPD primers was much higher than that of the three ISSR primers (6), which suggested that the RAPD markers were superior to ISSR markers in the capacity of revealing more informative bands in a single amplification. The similar results were observed by Fernández et al. [11] and Tanyolac [10].

Due to its worldwide distribution, the valuation of the genetic diversity among barley germplasm from different countries has been performed [7, 9, 11, 22–24]. Bernard et al. [15] analyzed the genetic diversity in 88 genotypes from 20 populations of wild barley from Israel, Turkey, and Iran by RAPD markers. When the total genetic diversity were estimated, 75% of the variation detected was partitioned within the 88 genotypes and 25% among the populations. When variation between countries was assessed, no substantial differences were found, because most of the variation detected (97%) was partitioned within the 20 populations and the remainder among the countries. Therefore,

Table 3: Polymorphism exhibited by ISSR and RAPD primers in barley.

	Primers	Tm (°C)		Total bands	Polymorphic	(%) Polymorphism	Resolving power (Rp)	Average of informativeness bands (AvIb)
		Theoretical	Optimal					
ISSR	UBC-890	56.39	56	3	2	66.67	1.16	0.39
	UBC-888	56.39	55	3	2	66.67	0.86	0.29
	A12	55	55	3	2	66.67	0.74	0.24
	Total	—	—	9	6	—	—	—
	Mean	—	—	—	—	66.67	0.92	0.30
RAPD	UBC-402	55	47	4	4	100	1.90	0.47
	OPA-11	50	47.5	5	5	100	1.89	0.37
	BY-15	32	34	8	8	100	5.20	0.65
	total	—	—	17	17	—	—	—
	Mean	—	—	—	—	100	2.99	0.49

Rp: Resolving power.
Ib: Band informativeness.

Table 4: Different classes obtained by dendrogram clustering using ISSR data.

Cluster	Specimens code
C1	1
C2	2, 3, 23, 49
C3	4, 17, 56, 59, 66
C4	5, 6, 7, 8, 14, 15, 16, 18, 26, 31, 45, 50, 51, 52, 62, 71, 72,74
C5	9, 10, 11, 12, 19, 20, 21, 22, 24, 25, 28, 29, 30, 32, 34, 35, 36, 37, 38, 39, 40, 41, 42, 44, 46, 48, 73, 75, 77
C6	13
C7	27, 33, 58
C8	43
C9	47, 57
C10	53, 54, 63, 65, 69, 76
C11	55
C12	60
C13	61, 64
C14	67
C15	70
C16	78
C17	79
C18	80

Table 5: Different classes obtained by dendrogram clustering using RAPD data.

Cluster	Specimens code
C1	1, 2, 3, 5, 6, 8, 9, 10, 11, 1214, 15, 16, 20, 21, 22, 23, 24, 25, 26, 28, 31, 34, 35, 40, 42, 43, 44 73, 75, 49, 50, 51, 53, 56, 30, 52, 54, 27, 62 65, 66, 72, 76 32, 60, 69, 71, 58, 59 4, 41, 37, 77
C2	61, 64
C3	7, 17, 18, 19, 29 36, 33, 38, 39 40, 63, 78 79 80, 48, 45
C4	55
C5	70
C6	13, 68
C7	47, 57
C8	67
C9	69

Table 6: Comparison of polymorphism detected by RAPD and ISSR markers in 80 barley specimens.

	Average band/Primer	Average polymorphic band/Primer	Correlation RAPD/ISSR
RAPD	5.66	5.66	
ISSR	3	2	0.12

the barely specimens were closed together independently of their geographic origin. In this study, both dendrograms based on RAPD and on ISSR markers do not show geographic profiling between barely specimens (Figures 1 and 2). Moreover, it has been reported that the dendrogram generated by the ISSR matrix agrees better with the genealogy and the known pedigree of the barley cultivars than the dendrogram generated by the RAPD results [11]. On the other hand, it has been found that the data based on RAPD-GS were more correlated with the geographic distribution of the genus *Houttuynia thunb*, while the data based on ISSRs were closely related with their number of chromosomes [8]. It could be partially explained by the different number of informative PCR products (84 for RAPDs and 105 for ISSRs). They reinforced again the importance of the number of loci and their coverage of the overall genome and obtained reliable estimates of genetic relationship among the studied materials [11].

The microsatellites or intersimple sequence repeat (ISSR) markers and randomly amplified polymorphic DNA

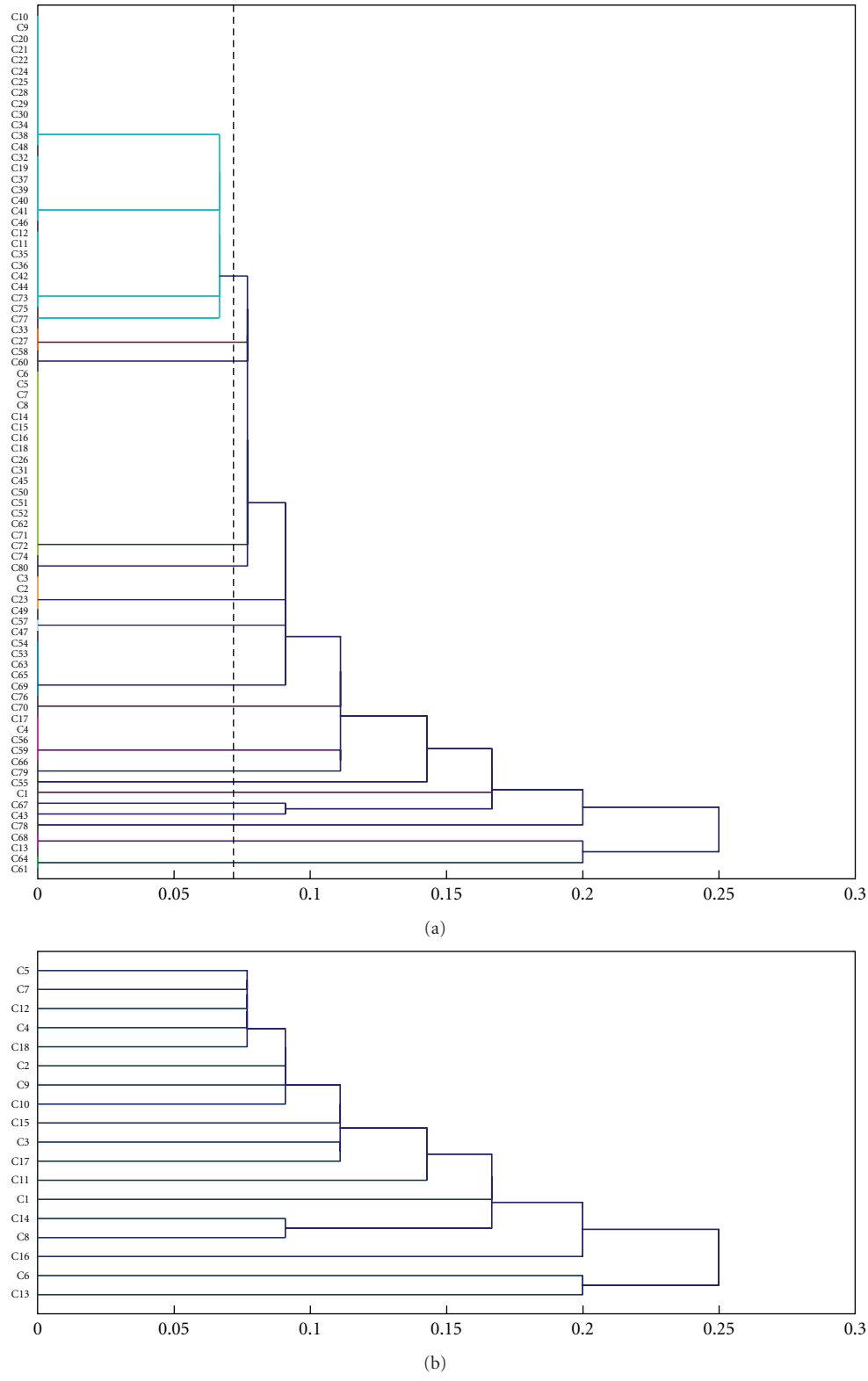

Figure 1: Dendrogram clustering revealed by 80 barley specimens (a) and clusters (b) using ISSR data and constructed based on the Dice dissimilarity index.

(RAPD) markers have proved to be the most polymorphic markers in barley and hence are highly useful markers for various applications in barley [11]. Apart from using them in diversity analysis, ISSR markers have been showed to be associated with various agronomically important traits, namely, dwarfing and vernalization response [25], leaf rust resistance [26], kernel hardness [27], cadmium uptake [28] preharvest sprouting tolerance [29], protein content [30]

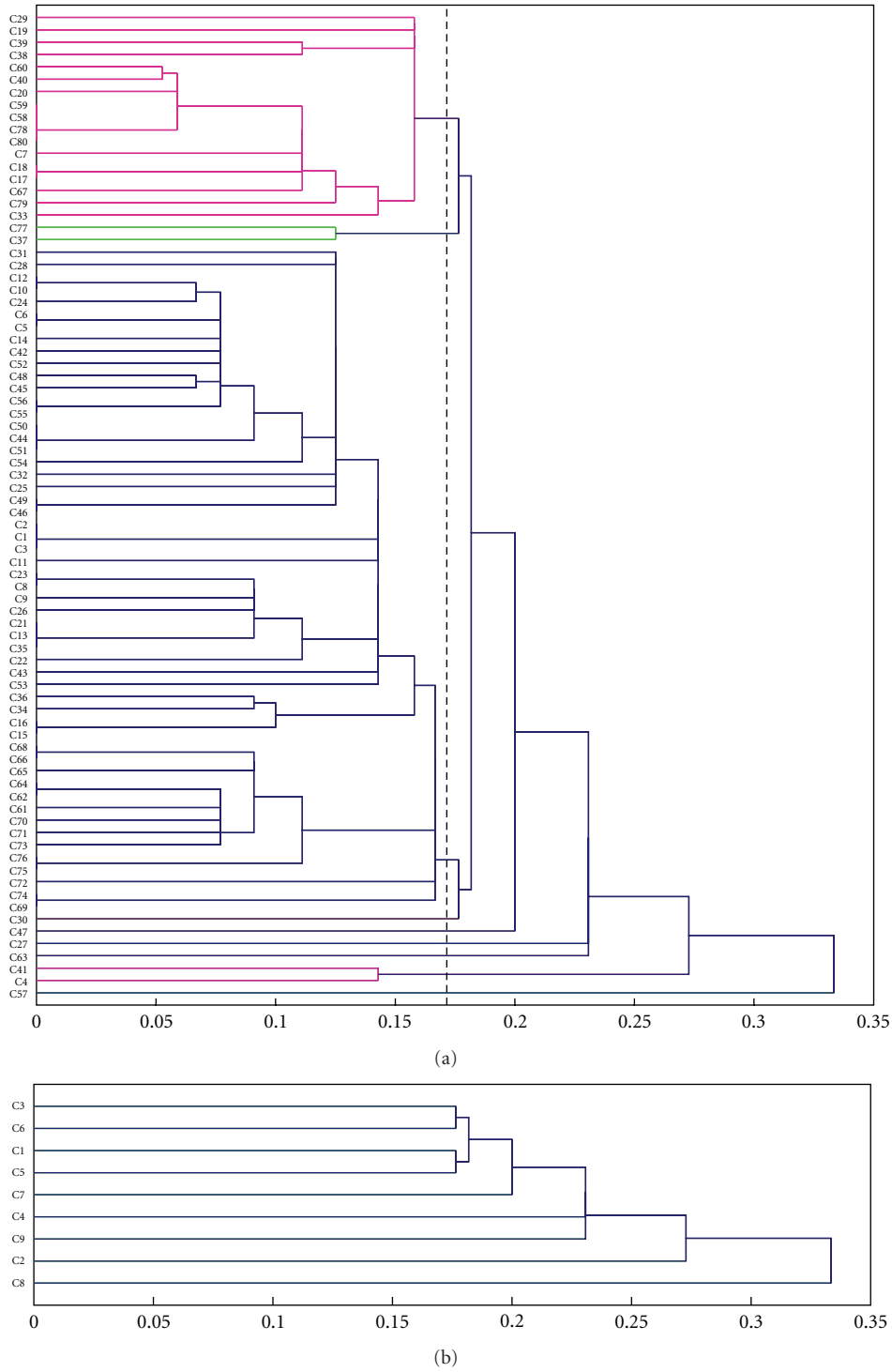

Figure 2: Dendrogram clustering revealed by 80 barley specimens (a) and clusters (b) using RAPD data and constructed based on the Dice dissimilarity index.

resistance to common bunt [31], powdery mildew resistance [32], kernel traits [33], flour viscosity [34]. RAPD markers were also shown to be associated with various traits such as the *Aegilops speltoides* leaf rust resistance gene Lr 28 in wheat [35], various traits contributing to kernel hardness in bread wheat [36], and cadmium intake in durum wheat [28]. These markers can be used for selection of important agronomic traits which would increase the efficiency and precision of breeding. In a previous study [37], some traits were used to evaluate the agronomical potentiality of barely specimens in south Tunisia. It has been noted that although the importance of agronomic parameters, it is necessary to use other markers to study diversity and select genotypes with high potential.

Comparing our results with other work on the study of molecular polymorphism in barley by RAPD markers and using the same primers, we deduced differences in the number and size of bands. Among the primers used by Owuor et al. [38], to study the polymorphism in wild barley (*Hordeum spontaneum*) in Palestine, it has been noted that the OPA-04 and OPA-18 primers were also used for the study of diversity in Morocco barley specimens [39]. The UBC-534 and UBC-490 primers were used to study the diversity of Palestine barley specimens [40].

According to molecular analysis, the geographical origin of specimens has no influence on the clusters obtained and seems to favor the hypothesis of the existence of a common origin for all barley cultivars. It has been assumed that the wild barley (*Hordeum vulgare* ssp. *spontaneum*) Tibet is the ancestor of cultivated barley worldwide [41].

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