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Full Length Research Paper

Morphological and molecular characterization of six of the most frequently cultivated hard wheat varieties in Tunisia

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To effectively differentiate and characterize six of the most cultivated hard wheat (*Triticum durum* Desf) varieties in Tunisia, morphological and molecular markers were used. In the former approach, 10 qualitative characters were employed, as recommended by UPOV descriptors. Twelve of the 17 screened simple sequence repeat (SSR) primers showed clear polymorphic patterns following agarose and polyacrylamide gel electrophoresis. Of these 12 primers, 64.3% produced polymorphic bands. Based on pedigree and origin, it was concluded that there was a narrow genetic base between these varieties. Mantel's test showed a weak correlation between morphological and molecular data (r = 0.24), indicating that these two approaches are complementary and can be used to study polymorphism, even within a small durum wheat collection. This data set is important for Tunisian hard wheat breeders who would seek to expand the genetic base of their breeding material.

Key words: Simple sequence repeat (SSR), International Union for the Protection of New Varieties of Plants (UPOV) descriptor, hard wheat, polymorphism.

INTRODUCTION

Cereal grain production is a strategic sector in Tunisian agriculture. Recently, increasing attention has been paid to the research of new genotypes, which led to the registration of more than 50 new varieties (Deghais et al., 2007). These varieties were developed either by crosses between Tunisian varieties (for example, 'INRAT69', 'Razzek', 'Maali', 'Om Rabia', 'Nasr'), through varietal exchange between research institutions ('Kyperounda', 'Kavkaz', 'Achtar', 'Nesma', etc.) or through adaptation of descendants native to CIMMYT ('Maghrebi', 'Karim', 'Khiar') and ICARDA ('Om Rabia', 'Nasr'). Morpho-physiological characterization is most commonly used in polymorphism research. Morphological characterization of species and/or of varieties used to be the only means to identify plants (Abdellaoui et al., 2007; Haljak et al., 2008; Andreini et al., 2009). Indeed, criteria established by the International Union for the Protection of New Varieties of Plants (UPOV) or by IPGRI were so finite and defined that they are now used the world over. Thus, in this study, we tried to characterize our varieties on the basis of the UPOV criteria (UPOV, 1988).

Several molecular markers such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) (Medini et al., 2003; Üncürlü et al., 2001), simple sequence repeat (SSR) (Dograr et al., 2000; Eujayl et al., 2002; Pagnotta et al., 2004; Zarkti et al., 2010; Golabadi et al., 2011), expressed sequence tag (EST)-SSR (Wang et al., 2007; Leigh et al., 2003; Maccaferri et al., 2003; Diab et al., 2012), inter simple sequence repeat (ISSR) (Carvalho et al., 2000; Mondini et al., 2010), and nucleotide-binding sites have been used to differentiate and categorize different varieties of durum wheat (Dograr et al., 2000; Eujayl et al., 2002; Medini et al., 2003).

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Table 1. Characteristics of six varieties of hard wheat, the most cultivated in Tunisia.

Variety	Origin: cross/selection	Pedigree	Pedigree of some parents in common of studied varieties	Date of registration
'Razzak'	INRAT/INRAT	Karim/Dmx69-331	Pedigreeof 'karim': 21563-AA "S" X Fg "S"	1987
'Maâli'	INRAT/INRAT	CMH80A.1016/4/TTURA/CMH77//CMH77.774/3/YAV79/5/Razzak/6 /DACK "S" / YEL "S"//Khiar Lignée D92-27-11Bj-OBj-9Bj-1Bj-OBj	Pedigree of 'Razzak': Karim/Dmx69-331	2007
'Karim'	CIMMYT-Mexico/INRAT	21563-AA "S" X Fg "S"	Pedigree of 'Fg' "S" : Jo"S"/3/61.130/LDS//GLL"S"	1982
'Khiar'	CIMMYT-Mexico/INRAT	Chen "S"/Altar 84 CD57005-1Y-2B-5Y-1M-0Y-0Bj	Pedigree of 'Chen': "SHWA"S"/BYE*2/TC60//TAC125E/3*TC60/3/61.130/LDS//GLL "S"	1992
'Om Rabia'	ICARDA -Syria	Jori C69/Hau LO589-4L-2AP-0AP-Obj	Pedigree of 'Jori C69': BYE*2/TC60//TAC125E/3*TC60	1996
'Nasr 99'	ICARDA	GdoVZ512/Cit/Ruff/Fg/3/Pin/Gre //TrobICD85-1340-ABL-6AP-0TR-10Bj-3Bj-0Bj	Pedigree of 'Gre': GS"S"/CR"S"/3/GO"S"/AA"S"/CIT"S"	2003

According to Brajcich et al. (1986): (/) cross giving hybrid F1, (-) in the old system; (//) a cross between F1 and another parent, (/) in the old system; (*) represents a backcross; ("S"): Soeur (sister). Numbers indicate the dosage of the recurrent parent for example 2 and 3.

This study uses morphological and molecular methods to characterize six varieties of hard wheat, the most commonly cultivated in Tunisia. The ability of morphological and molecular markers to reveal their polymorphism is assessed.

MATERIALS AND METHODS

Plant material

The seed of six varieties of hard wheat ('Karim', 'Khiar', 'Mâali', 'Nasr', 'Razzak' and 'Om Rabia') were provided by INRAT (National Institute of the Agronomical Research of Tunisia). Table 1 summarizes some characteristics of these varieties. Two sowing methods were used. The first one was carried out in pots with five seeds/pot. Seedlings derived from these seed were used to extract DNA. The second sowing method was carried out in the field. Each variety was sown along 2 m-long rows at a density of 40 seeds/line with 5 cm between seeds. This plot was used to study morphology from the seedling stage to maturity.

Morphological study

Evaluation of qualitative characters

The morphology of plot-grown plants was described on the basis of terminology recommended by UPOV (1988). The most informative characters were selected among 31 characters and evaluated from 10 plants/variety. These characters were related to grain (shape, color), ear [density, shape in profile view (that is, tapering, parallel-sided, semi-clavate, clavate, fusiform)], hairiness of the margin of the first rachis segment), lower glume (shape, shoulder shape and width, length of beak), anthocyanin pigmentation and growth habit.

Data analysis

The quantitative characters (Table 2) were analyzed by STATITCF (version 5) using Fischer's F-test at $P < 0.05$ (Beaux et al., 1991). The analysis was completed with multiple comparisons between means using Newman and Keul's test (Steel and Torrie, 1980; Dagnélie, 1986) at $P < 0.05$ showing the different classes of varieties according to quantitative characters (that is, showing diversity between genotypes). Phenotypic or qualitative characters (Table 3) were translated into a matrix with a double entry with the aid of MVSP software (version 3.1p). Using Kovach computing services (http://www.Kovocomp.com), it was possible to generate a dendrogram on the basis of the UPGMA (unweighted pair group method with arithmetic averages) method.

Molecular study

DNA extraction

The genomic DNA of all varieties was extracted from young leaves following a CTAB method modified and described by Ben Naceur (1998), and followed by an organic extraction in chloroform: isoamyl alcohol (24:1). DNA was purified by RNase (10 µg/ml) and its concentration was estimated using 0.8% agarose gel. DNA was dissolved and conserved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0.

Primers used in PCR

A total of 56 RAPD primers were tested, although only 20 showed polymorphic profiles (OPB3, OPK1, OPM2, OPF9, OPA2, OPC7, OPJ1, OPI1, OPK9, OPA7, OPN7, OPC15, OPA5, OPI4, OPE18, OPF4, OPG12, OPM8, OPI20, OPD20) while 5 showed no amplification (OPD10, OPB13, OPP18, OPG1, OPL6) and 31 showed monomorphic profiles. A total of 17 SSR primers were tested, although only 12 (WMC 283, WMC21, WMC19, WMC23, WMC48, WMC22, WMC24, WMC50, WMC27, WMC16, WMC13, WMC15) produced polymorphic bands (Table 4).

DNA amplification

Amplification was performed using a thermocycler (Biometra Uno II), in a total volume of 25 µl containing 2 mM $MgCl_2$, 1 mM dNTP, 2.5 µM of forward and reverse primers, 1 U of *Taq* DNA polymerase (Promega; http://www.promega.com), 1X buffer and 50 ng/µl DNA. PCR consisted of one round of predenaturation at 94°C for 3 min followed by 35 amplification cycles of: 1 min

Table 2. Analysis of variance for quantitative characters.

Quantitative character (variety effect)	SS	DF	Mean square	F(1)	P-value
Number of tiller (C1)	156.88	5	31.38	1.36	0.2516
Plant length (C2)	6575.75	5	1315.15	41.15	0
Leaf lenght (C3)	914796160	5	182959232	278.93	0
Leaf width (C4)	94.68	5	18.94	2.82	0.0245
Leaf area (C5)	127923488	5	25584698	5.03	0.0008
Number of seeds/ear (C6)	28380.40	5	5676.08	28.57	0
100-seed weight (C7)	3.44	5	0.69	0.11	0.9878

(1) = Fisher's test (5%).

Table 3. Phenotypic characters studied.

C8 / coleoptile: anthocyanin coloration.	**C21 / Lower glume: shoulder width.**
C9 / first leaf: anthocyanin coloration.	**C22 / Lower glume: length of beak.**
C10 / plant: growth habit.	C23 / Lower glume: shape of beak.
C11 / flag leaf: glaucosity of sheath.	C24 / Straw: pith in cross section (half-way between base of ear and stem node as follows):
C12 / flag leaf: glaucosity of blade.	C25 / Awn: color.
C13 / awn: anthocyanin coloration.	**C26 / Ear: length excluding awns.**
C14 / Culm: hairiness of uppermost node.	**C27 / Ear: hairiness of margin of first rachis segment.**
C15 / Culm: glaucosity of neck.	C28 / Ear: color (at maturity).
C16 / Ear: glaucosity.	**C29 / Ear: shape in profile view.**
C17 / Ear: distribution of awns.	**C30 / Ear: density.**
C18 / Awns at tip of ear: length in relation to ear.	**C31 / Grain: shape.**
C19 / Lower glume: shape (spikelet in mid-third of ear).	**C32 / Grain: coloration.**
C20 / Lower glume: shape of shoulder.	**C33 / Seasonal type.**

Characters in bold showed the difference between varieties

denaturation at 94°C, 1 min hybridization at 55°C, 1 min of extension at 72°C. These cycles were followed by a final extension for 2 min at 72°C (Chaabane et al., 2009). PCR products were separated on a 2% agarose gel. To better discern some fine bands, we also used a 40% polyacrylamide gel prepared with 49 ml distilled water; 7 ml TBE (10X); 14 ml of 40% acrylamide (19:1; acrylamide: bisacrylamide); 560 µl PSA (ammonium persulfate (10X) and 6 µl TEMED (tetramethylethylenediamine). Bands were visualized with a GelDoc imaging system with a UV filter (570 to 640 nm).

Data analysis

Electrophoretic profiles generated by SSR primers were examined by simple visual observation where the presence (1) or absence (0) of bands was used to establish a binary matrix composed of 0 and 1. From this matrix, a dendrogram depicting genetic distances was created using TREECON software for Windows (v 1.3b) on the basis of the UPGMA method and the dissimilarity coefficient of Nei and Li (1979). To estimate the statistical robustness of the topology of the dendrogram derived from SSR primers, we used a bootstrap method (Felsenstein et al., 1985) with 100 replications. This test was also accomplished with TREECON software (v 1.3b). The degree of polymorphism generated by a primer provides information about its aptitude to differentiate varieties, indicated by polymorphism information content (PIC) (Table 5), which was calculated by the equation given by Botstein et al. (1980) and Anderson et al. (1993):

$$PIC = 1 - \sum_{j=1}^{n} P_{ij}^2$$

PIC is the polymorphic information content of marker i whereas Pij is the frequency of the jth pattern for marker i with the summation extending across n patterns.

RESULTS AND DISCUSSION

Morphological study

The similarity dendrogram obtained with MVSP software by using morphological data allowed all varieties to be categorized (Figure 1). On the basis of the similarity coefficient (SC = 90%), varieties were classified into three distinct groups: group 1 ('Khiar', 'Karim'), group 2 ('Nasr', 'Razzek', 'Mâali') and group 3 ('Om Rabia'). By examining the history, origin and pedigree of these varieties, we noted that the varieties of group 1, which showed a high SC = 93.49%, were both bred in CIMMYT and have common parents (Table 1). 'Razzek' and 'Mâali', which regrouped together with a high SC = 92%

Table 4. Description of tested SSR primers (http:www.wheat.pw.usda.gov/ggpages/SSR/WMC).

Primer	Sequence	Position on chromosome	Motif
WMC23F	5′-ATT CgC TCA TAC gAT Agg gTT g-3′	-	(CT)22 (CT)18
WMC23R	5′- AgA ggC Tgg TgT AgT Tgg TTT g-3′		
WMC25F	5′- TCT ggC CAg gAT CAA TAT TAC T -3′	2B	(GT)26
WMC25R	5′- TAA gAT ACA Tag ATC CAA CAC C -3′		
WMC26F	5′- TgT ATT CCC ATg AAC CCC ACT g-3′	-	(CA)14(CA)9(CAA)20
WMC26R	5′- TCT CCC AAT TgA TTT ggA Agg C -3′		
WMC27F	5′- AAT AgA AAC Agg TCA CCA TCC g-3′	2B; 5B	(GT)25
WMC27R	5′- TAg AgC Tgg AgT Agg gCC AAA g-3′		
WMC24F	5′- gTg AgC AAT TTT gAT TAT ACT g -3′	1A	(GT)28
WMC24R	5′- TAC CCT gAT gCT gTA ATA TgT g-3′		
WMC48F	5′- gAg ggT TCT gAA ATg TTT TgC C-3′	4B	(GA)9
WMC48R	5′- ACg TgC TAg ggA ggT ATC TTg C-3′		
WMC50F	5′- CTg CCg TCA ggC CAg gCT CAC A-3′	3A	(GT)10(GT)16
WMC50R	5′- CAA CCA gCT AgC TgC CgC CgA A-3′		
WMC283F	5′- CgT Tgg CTg ggT TAT ATC ATC T-3′	4A	(CA)19(CA)8
WMC283R	5′- gAC CCg CgT gTA AgT gAT Agg A-3′		
WMC13F	5′- gTg TCg gAT gCg CgC gAT AgA AT-3′	-	2(CT)
WMC13R	5′- ATg CAT AAA ACg CgA CCT CCC CT -3′		
WMC15F	5′- AgT CCg ATT Cgg ACT CCT CAA g-3′	4A	(CA)(CT)
WMC15R	5′- ggA CTA ACC gAg ggT AgT TCA g-3′		
WMC16F	5′- ACC gCC TgC ATT CTC ATC TAC A -3′	4B	(CT)
WMC16R	5′- gTg gCg CCA Tgg TAg AgA TTT g-3′		
WMC17F	5′- ACC TgC AAg AAA TTA ggA ACT C-3′	7B-7A	(CA)
WMC17R	5′- CTA gTg TTT CAA ATA TgT Cgg A-3′		
WMC18F	5′- CTg ggg CTT ggA TCA CgT CAT T-3′	2D	(CA)(CT)
WMC18R	5′- AgC CAT ggA CAT ggT gTC CTT C-3′		
WMC19F	5′- CTg ACA TgC ggC ATT CAC TTC C-3′	-	(CA)
WMC19R	5′- Agg CTT AgA ACA CAC CgA CAC g-3′		
WMC20F	5′- TTA AAA ACA CgC ggA TCT TCT C-3′	-	(CA)
WMC20F	5′- gTA CTC ACA TAT TTC TCg gTC T-3′		
WMC21F	5′- CgC TgC CgT gTA ACT CAA AAT C-3′	-	(GA)37
WMC21R	5′- AgT TAA TTg ggC gCT CCA AgA A -3′		
WMC22R	5′- ATC ATT ggT TTC CTC TTC ACT T-3′	-	(GT)24
WMC22R	5′- gTg gAC TAT TTA ACA TCT TCA T -3′		

Table 5. PIC generated by the different polymorphic primers.

Primer	PIC	Primer	PIC
WMC283	0.87	WMC 50	0.97
WMC21	0.94	WMC27	0.96
WMC19	0.91	WMC16	0.53
WMC24	0.99	WMC13	0.71
WMC48	0.87	WMC15	0.72
WMC22	0.84	WMC23	0.99

also shared the same parents. In fact, ‘Razzek’ is one of ‘Mâali’s parents. The presence of ‘Nasr’ in the same group (group 2) is because of some morphological characters which they have in common: no anthocyanin pigmentation, erect growth habit, distribution of awns on the entire length of the ear, elongated spikelet in mid-third ear, hairiness of the uppermost node, glaucosity of the neck and a black awn. ‘Om Rabia’, bred in ICARDA, could

be differentiated from the other varieties and had a weak SC = 88.7%. Ben Naceur et al. (2001) also discriminated 'Om Rabia' from other varieties using physiological features only, and noticed a particularly unique physiological character in this variety: a delay in ear emergence but a speed of grain-filling which allows the plant to avoid drought at the end of the cycle. Other qualitative characters contributing to its differentiation from other varieties are: stem height (taller than other varieties), strong hairiness of the margin of the first rachis segment and the medium length of the beak of the lower glume.

On the basis of this morphological study, we could explain the regrouping of varieties in accordance with information given by their pedigree. However, a study done by Maccaferri et al. (2007) on 58 durum wheat (*Triticum durum*) accessions showed that, in spite of the large number of morphological markers studied, information generated by such markers did not succeed in bringing the varieties closer to the information of the pedigree. Other studies done by Rebourg et al. (2001) and Heckenberger et al. (2005a, b) on corn show that only 26 and 25 phenotypic markers, respectively, are enough for characterizing genotypes caused by the accuracy of information provided by morphological characterization. Carvajal-Rodriguez et al. (2005) and Boudour et al. (2011) noted that morphological characters are sensitive to environmental changes and that studies of polymorphism based on qualitative phenotypic characters are deficient and need to be supplemented with molecular studies. Chaabane et al. (2012) showed the importance of molecular studies to supplement morphological analyses to check for salt tolerance in durum wheat, although, Farshadfar et al. (2012) used *in vitro* studies to supplement *ex vitro* studies using only morphological markers to reach the same conclusions.

Molecular study

In this study, we tested 17 primer pairs, 12 of which (WMC24, WMC22, WMC48, WMC19, WMC21, WMC283, WMC50, WMC27, WMC23, WMC15, WMC13, WMC16) generated polymorphic profiles. Three primers (WMC17, WMC18, WMC20) generated monomorphic profiles, whereas two primers (WMC25, WMC26) generated no bands. Of the 12 primers, 64.3% generated polymorphic bands. Based on PAGE and AGE (Figure 2 (A to I)), we discovered 253 polymophic bands for the 12 primers, which translates into 21.03 bands per gel or 3.51 bands per variety, which is a usual number (Panwar et al., 2010). The majority of bands were between 100 bp and 1 Kbp (Figure 2). For every primer, we calculated the degree of polymorphism (PIC). PIC values varied from 0.53 to 0.99, implying the large ability of primers to detect polymorphism. In comparison to Ethiopian material, the low PIC value in Tunisian material was higher than the minimum (0.14 to 0.92) values found by Mondini et al. (2010) in Ethiopia hard wheat. The discrepancy between the number of bands/primer pair and the wide range often observed within the literature is often attributable to "stutter" bands (Park et al., 2009). The dendrogram of genetic dissimilarity generated by molecular data (Figure 3) allowed us to categorize the varieties, and to show the ability of PCR-based SSR markers to assess diversity in Tunisian durum wheat as effectively as in other crops as maize (Taramino and Tingey, 1996) and rice (Zhao and Kochert, 1993). The SC of 'Karim' was 30, representing a single group while 'Khiar', 'Om Rabia', 'Razzek', 'Maâli' and 'Nasr' formed another group.

When the history and pedigree of the varieties studied was examined, some groups were shown to have common parents. In fact, 'Maâli' is one of 'Khiar's parents, which explains their regrouping with a high degree of genetic similarity (87%). The presence of 'Om Rabia' in the same sub-group as 'Maâli' and 'Khiar' can be explained by the presence of some common ancestors (BYE*2/TC60//TAC125E/3*TC60) and the level of the pedigree of 'Om Rabia' and 'Khiar's parents (Table 1). 'Nasr' and 'Razzak' formed a sub-group in which two common ancestors were present ('Flamingo' and 'Anhinga' sister) (Table 1). Thus, descendants from international cereal centers (ICARDA or CIMMYT) are genetically close and have at least a common parent used to improve resistance to some biotic or abiotic stresses. That resulted in a dendrogram with a narrow genetic base. In general, cophenetic correlation coefficients above 0.70 are considered to be efficient for the graphical representation of contrasts between genotypes (Vieira et al., 2007). In fact, bootstrap analyses with 100 repetitions showed that all knots had upper (high) values (>50%), which confirms the robustness of the topology of the dendrogram. This result is confirmed by other studies related to corn (*Zea mays* L.) in which the matrix of cophenetic distances between lines in the UPGMA dendrogram was estimated at 0.601, confirming the reliability of the groups based on the UPGMA algorithm (Ribeiro et al., 2010).

In another study carried out to describe the genetic relationships among white oat (*Avena sativa* L.) genotypes, a bootstrap value of 82.7% created a consistent group based on molecular diversity and clustered genotypes more closely when molecular and morphological diversity were considered simultaneously (Benin et al., 2008). To compare results from molecular and morphological markers, we conducted a Mantel test (1967) by using the NTSYS program (version 5.1) (1997). The test shows that the correlation between the similarity matrix generated by morphological data and that generated by molecular (SSR) data was 0.24. The weak correlation between these two types of markers might be due to the nature of markers used which are dominant (De Vienne, 1998) in the case of morphological characters and codominant in the case of microsatellites

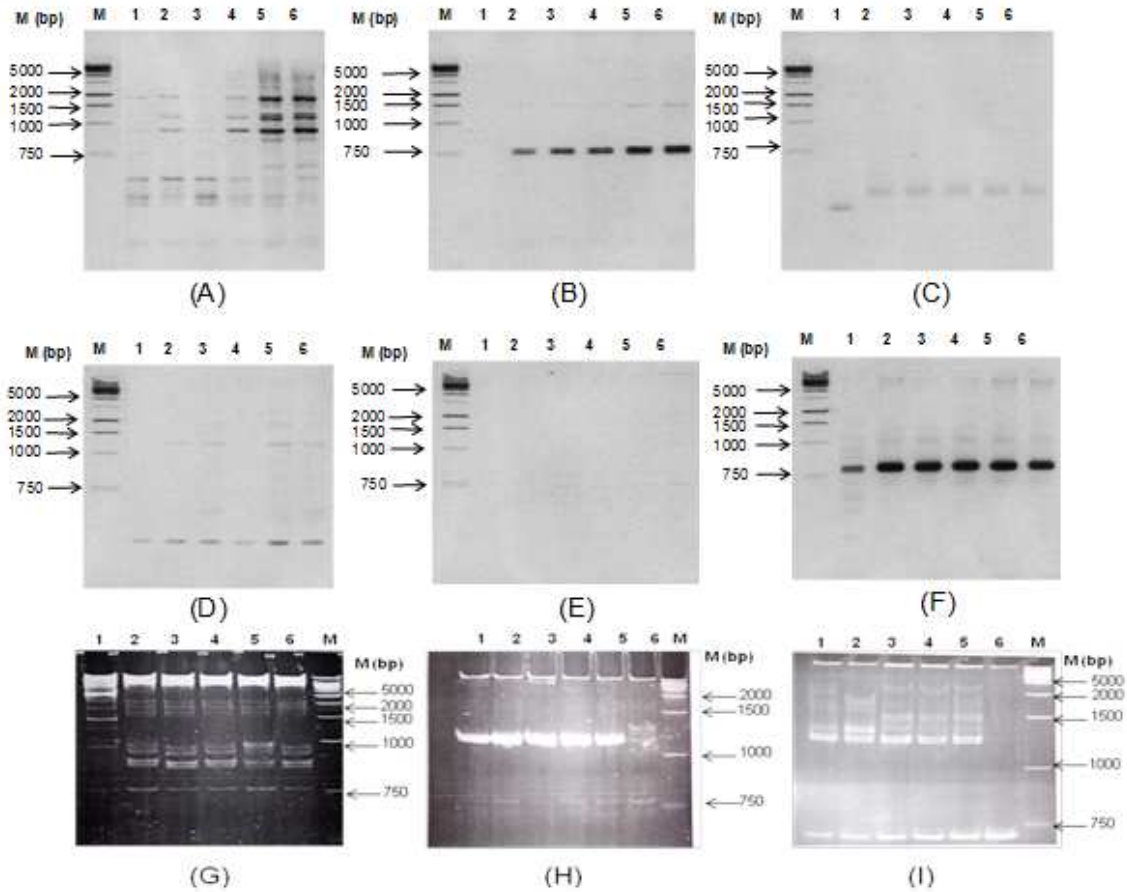

Figure 2. Agarose gel showing the allelic segregation of WMC 14 (A), WMC 15 (B), WMC 16 (C), WMC 21 (D), WMC 17 (E) and WMC 23 (F). Polyacrylamide gel showing the allelic segregation of the WMC 50 (G), WMC 24 (H), WMC 48 (I), SSR markers in six analysed varieties: 1: Karim, 2: Khiar, 3: Maâli, 4: Nasr, 5: Om Rabia, 6: Razzak. M: molecular size standard (1-Kb DNA ladder).

(Ribeiro et al., 2010). Roy et al. (2004) and Nevo (1995) reported an extremely low correlation (0.072) between genetic distances estimated for wheat using AFLP markers and 14 morphological characters, indicating an association close to null. Maric et al. (2004) also reported a small correlation (r = 0.12) between distances estimated using RAPD markers and 12 morphological characters for hexaploid wheat cultivars. Karanja et al. (2009) showed, in maize, that the correlation between molecular and morphological matrices was low (r = 0.232). Benin et al. (2008) also showed a low correlation (r = 0.33) between both markers to describe white oat genotypes.

The weak correlation between morphological and SSR (that is, molecular) markers suggests that these analyses are subjected to diverse types of errors. For example, when we make a comparison, we compare two comparable things, but in this case, one is dominant while the other is codominant, that is, an inherent difference exists. Another example, in this type of study, is that the entire genome can be probed with molecular markers while morphological markers only allow us to describe a few and precise characters visible to the naked eye. The correlation observed between markers (morphological versus molecular) differs from study to study and on the plant material and technology used (Maccaferri et al., 2007). Indeed, the correlation between RFLP and morphological characters, as determined by Autrique et al. (1996) for various varieties of hard wheat was 0.47. This value doubled that observed in our study, may be due to the difference between morphological traits and molecular (SSR) markers; a hypothesis explained in details by Vahabi et al. (2008), who compared different morphological traits and SSR markers in *Plantago ovata*. In barley, the correlation between SSRs and morphological characters was not significantly different (Russell et al., 2000).

Conclusions

Despite divergences between molecular and phenotypic

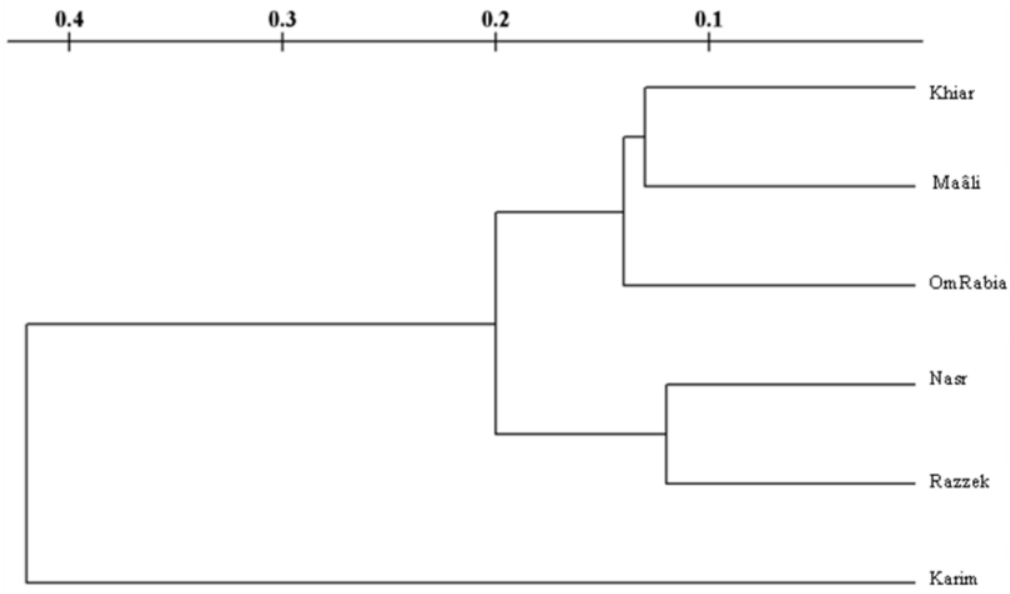

Figure 3. Dendrogram showing relationship among 6 varieties of hard wheat generated by SSR markers using Treecon software.

classifications, the analysis of phenotypic variation allows us to approach the variation of morpho-phenological characters of plants better than molecular markers. In fact, these complex quantitative and sensitive characters to environmental variation provide us with information on the relations between constraints and the capacity of populations to adapt (Carvajal-Rodriguez et al., 2005). Morphological traits are usually associated with a number of limitations, including low polymorphism, low heritability and vulnerability to environmental changes (Smith and Smith, 1992). Although, there are disadvantages associated with phenotypic markers, they continue to play a major role in studying and characterizing germplasm since they require no expansive laboratory facilities or procedures. However, despite the utility of morphological markers, molecular markers remain the most required in studies of polymorphism among a limited number of genotypes. Genetic assessment has been greatly facilitated by molecular markers, which are good alternative methods mainly characterized by their rapidity, to detect polymorphism between different systematic levels. Moreover, molecular markers are not influenced by environment factors like morphological traits (Smith and Smith, 1989) and the genetic polymorphism observed using molecular markers may provide information on the history of cultivars, but it does not necessarily reflect what may be observed with respect to agronomic traits (Métais et al., 2000). Hence, no marker can solely give all the information needed in plant adaptation, breeding, evolutionary and conservation programmes without support from another technique (Taramino and Tingey, 1996).

This study shows that both morphological and molecular markers need to be studied concurrently to obtain better knowledge about dissimilarities among varieties that share parents, but that this is possible even when a limited number of genotypes is used, six in our case.

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