Scientia Agricola
http://dx.doi.org/10.1590/0103-9016-2015-0054

Genetic diversity reduction in improved durum wheat cultivars of Morocco as revealed by microsatellite markers

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Edited by: Leonardo Oliveira Medici

Received February 20, 2015
Accepted June 17, 2015

ABSTRACT: It has been argued that genetic diversity in crop varieties has been on the decline in recent times due to plant breeding. This can have serious consequences for both the genetic vulnerability of crops and their plasticity when responding to changes in production environments. It is, therefore, vital for plant breeding programs to maintain sufficient diversity in the cultivars deployed for multi-period cultivation. In this study, to understand the temporal genetic diversity in durum wheat, 21 improved durum wheat cultivars released in Morocco, since 1956 and five exotic cultivars currently used in crossing programs were analyzed using 13 microsatellite markers. The analysis revealed a total of 44 alleles and average genetic diversity of 0.485 with genetic distances ranging from 0.077 to 0.846 at 13 microsatellite loci in Moroccan durum wheat cultivars. All the durum cultivars of Morocco could be distinguished using the 13 microsatellite markers. The total number of alleles and unique alleles were highest in cultivars developed before 1990, decreasing in cultivars developed during the 1990s and 2000s, indicating that recent durum breeding efforts have reduced allelic richness in recent cultivars. Thus, deployment of exotic durum wheat lines in breeding programs could enhance genetic diversity in durum wheat cultivars.

Keywords: *Triticum turgidum* L. subsp. *durum*, cultivar characterization, SSR markers

Introduction

Durum wheat (*Triticum turgidum* L. subsp. *durum*, 2n = 4x = 28; AABB) is a tetraploid wheat, mainly grown in the Mediterranean basin, and other parts of the world for human consumption. Morocco produces around 1.2 million tons of durum wheat annually. However, most years Morocco imports durum wheat to supply its growing demand. Therefore, the improvement of such a crop in terms of yield and quality is necessary. The overall objective of Moroccan wheat breeding was and remains the development of durum wheat genotypes with high genetic potential for yield and quality. Efforts to improve durum wheat were initiated in 1921, through mass-selection and introduction of exotic cultivars. Variability was generated through hybridization between better performing local and exotic cultivars (Jlibene and Nsarellah, 2011). In all these periods, the introduced and improved varieties had a great impact on Moroccan wheat production.

Genetic variation in registered varieties is fundamental to the improvement of future breeding programs by providing a basis for selection of superior parental combinations and predictions of progeny performance (Haile et al., 2013). Analyses of genetic divergence and estimation of genetic distance between parents are useful for choosing parents in wheat hybridization programs (Islam, 2004). The loss of variation in crops due to the modernization of agriculture has been described as genetic erosion. Genetic erosion of cultivated diversity is reflected in a modernization bottleneck at diversity levels that occurred during the history of the crop (Wouw et al., 2009). It is crucial to formulate an idea about genetic diversity changes in existing gene-pools of cultivated crops in order to understand the impact of plant breeding on crop genetic diversity (Fu et al., 2005) and it could make crop improvement more efficient by the direct accumulation of desired alleles.

Molecular markers play a pivotal role in varietal evaluation; it can speed up the process and decrease the amount of plant material that needs to be screened in such experiments (Astarini et al., 2004). Microsatellite markers have been used for analysis of genetic diversity and identification of indigenous landraces and modern cultivars (Khanjari et al., 2007; Wang et al., 2007) and also used for temporal variation in wheat (Roussel et al., 2004 and 2005; Fu et al., 2006; Figliuolo et al., 2007; Huang et al., 2007; Fu and Somers, 2009). This study analyzed the use of microsatellite markers for cultivar genetic diversity analysis, genetic distance estimation and to understand temporal changes in genetic diversity and allele richness in Moroccan durum wheat cultivars developed since 1956.

Materials and Methods

Plant materials

A total of 26 durum wheat cultivars consisting of 21 improved cultivars released for cultivation in Morocco (Nsarellah et al., 2005) were provided by the National Gene Bank of Morocco, INRA, Settat, Morocco (Table 1). Five potential exotic cultivars which are important as donors in the breeding program, namely Vitron (Spain), Strong Field (Canada), Medora (Canada), Sceptre (Canada) and UC1113-GPC-B1 (USA) were also included in this study.

Table 1 – Improved durum wheat cultivars of Morocco procured from the National Gene Bank of Morocco[a].

Cultivar	Registration year	Pedigree
Kyperonda	1956	Selection from 'Cyprus' population
Cocorit	1975	Rae/4Tc60//STW63/3/AA'S'
Selbera	1982	Old landrace
Marzak	1984	Selection from CIMMYTs 'PYTII'
Karim	1985	Bittern 'S' same as JO'S'.AA':S'//FG'S'
Sebou	1987	Selection from 'Grebe'S' population.
Bel Bachir	1988	Improved cultivar introduced from Tunisia
Tensift	1988	Njoro 226 selection from unknown Cyprus population.
Oum Rabia	1988	Selection from 'Cyprus3' population.
Sarif	1988	Selection from the Lds/Mut//Teal'S' population
Jawhar	1993	Moroccan selection in hybridization with introduced material
Ourgh	1995	Moroccan crossing and selection of introduced material
Tarek	1995	Selection from a population derived from Moroccan line and introduced lines
Amjad	1995	Moroccan crossing and selection on introduced material
Merjana	1996	Selection from a population derived from crossing Moroccan line and introduced lines
Tomouh	1997	Selection on introduced material in Morocco
Marouane	2003	Crossing and Moroccan selection
Nassira	2003	Crossing and Moroccan selection
Amria	2003	Crossing and Moroccan selection
Irden	2003	Crossing and Moroccan selection
Icamor	2006	Crossing of parents from Morocco and ICARDA and selection at Morocco and ICARDA

[a]Kindly provided by Hassan Ouabbou

DNA extraction and microsatellites analysis

Genomic DNA was extracted from 4-week-old seedlings (5 cm of young leaf blades) of individual cultivars using the CTAB (cetyltrimethylammonium bromide) method of Saghai-Maroof et al., (1984) with minor modifications (Udupa et al., 1999): use of 2 % CTAB buffer for extraction instead of 1 % CTAB and use of sterile distilled water for dissolution of the final DNA pellet instead of 10 mM NH4OAc/0.25 mM EDTA (ethylenediaminetetraacetic acid). Quality and quantity of the isolated DNA were assessed by intactness and intensity of the DNA band, respectively, obtained after electrophoresis of 3 µL of the isolated DNA in 1 % agarose (w/v) gel, stained with ethidium bromide and visualized under Ultra Violet (U.V.) rays. The intensity of the band of isolated DNA was compared to known concentrations of lambda DNA digested with *Eco*RI and *Hin*dIII restriction enzymes.

Thirteen polymorphic microsatellites (Table 2) were used in this study. The Polymerase Chain Reactions (PCRs) were performed in total volume of 10 µL, containing 1x PCR buffer (1.5 mM $MgCl_2$), 200 µM of each dNTPs (deoxyribose nucleotide triphosphates), 10 pmoles of each primer, 0.5 U of *Taq* DNA polymerase and approximately 50 ng of genomic DNA. The amplification reaction was generated in the Eppendorf Master cycler with initial denaturation for five minutes at 94 °C, followed by 35 cycles of each cycle with 30 seconds denaturation at 94 °C, 30 seconds annealing at 59 °C, 45 seconds extension at 72 °C. Final extension was carried out at 72 °C for five minutes followed by cooling at 4 °C for an undefined period. Amplified products were separated on 6 % (w/v) denaturing polyacrylamide gels. The amplified bands were detected by silver staining. The size of each band was estimated simultaneously by means of a 100-bp DNA Ladder.

Data analysis

PowerMarker software (Ver. 3.0; Liu and Muse, 2005) was used to calculate genetic diversity, number of alleles and the shared allele genetic distance (Jin and Chakraborty, 1993). The average number of alleles, unique alleles and genetic diversity for each temporal group were calculated. These temporal groups were also compared with the exotic varieties currently used in breeding programs. To determine genetic divergence, genetic distances were calculated for each pair of temporal groups. A dendrogram was constructed based on genetic distance by using the Neighbor-joining (NJ) method (Saitou and Nei, 1987) and visualized using MEGA5 software (Tamura et al., 2011). An Analysis of Molecular Variance Analysis (AMOVA) and Principal Coordinates Analysis (PCoA) were undertaken using GenAlEx 6.5 software (Peakall and Smouse, 2012). The FPtest (Fu, 2010) was performed (with 50,000 random permutations) to test the significance of differences in allelic count between the temporal groups.

Results

Microsatellite polymorphism

A total of 44 alleles were detected for the Moroccan cultivars and 25 alleles for the 5 exotic cultivars

(Table 3). The number of alleles per locus ranged from 2 (*Xbarc263*, *Xwmc89*, *Xpsp2999* and *Xwmc24*) to 6 (for *Xgwm577*) with an average number of 3.38. The 13 microsatellites used were sufficient to differentiate all the 21 Moroccan cultivars and the five exotic cultivars. Average genetic diversity (*H*) calculated for all markers and Moroccan genotypes was 0.485 (Table 3). The *Xgwm136*, *Xgwm577* and *Xgwm389* markers were the most informative and showed the highest value (0.671, 0.626 and 0.621, respectively).

The 21 Moroccan durum wheat cultivars were discriminated using 13 microsatellite markers (Figure 1). The genetic distance (Table 4) was lowest between Amria and Irden, Marjana and Amjad, Sebou and Kyperonda, Bel Bachir and Vitron, Sarif and Vitron, and Tarek and Vitron (0.077), indicating that these accessions are closely related to each other. The highest genetic distance was observed between Karim and Kyperonda, Ourgh and Kyperonda, Ourgh and Sebou, and Marouane and Selbera (0.846). Cluster analysis based on the

Table 2 – Locus name, sequences, repeat motif of 13 microsatellite markers used in this study.

Locus	Location	Forward primer (5'-3')	Reverse primer (5'-3')	Repeat motif*	Reference
Xgwm33	1A	GGAGTCACACTTGTTTGTGCA	CACTGCACACCTAACTACCTGC	(GA)19	Röder et al., 1998
Xgwm389	3B	ATC ATG TCG ATC TCC TTG ACG	TGC CAT GCA CAT TAG CAG AT	(CT)14(GT)16	Röder et al., 1998
Xgwm146	7B	CCA AAA AAA CTG CCT GCA TG	CTC TGG CAT TGC TCC TTG G	(GA)5GG(GA)20	Röder et al., 1998
Xgwm397	4A	TGT CAT GGA TTA TTT GGT CGG	CTG CAC TCT CGG TAT ACC AGC	(CT)21	Röder et al., 1998
Xgwm136	1A	GAC AGC ACC TTG CCC TTT G	CAT CGG CAA CAT GCT CAT C	(CT)58	Röder et al., 1998
Xbarc263	1A	GGAAGCGCGTCAGCACTAGGCAAC	GGCTTCTAGGTGCTGCGGCTTTTGTC	(ATT)17	Ward et al., 2003
Xgwm130	7A 2B 7B	AGC TCT GCT TCA CGA GGA AG	CTC CTC TTT ATA TCG CGT CCC	(GT)22	Röder et al., 1998
Xwmc89	4A	ATGTCCACGTGCTAGGGAGGTA	TTGCCTCCCAAGACGAAATAAC	(CA)19 or (CT)8	Somers et al., 2004
Xgwm193	6B	CTT TGT GCA CCT CTC TCT CC	AAT TGT GTT GAT GAT TTG GGG	(CT)24imp(CA)8	Röder et al., 1998
Xgwm273	1B	ATT GGA CGG ACA GAT GCT TT	AGC AGT GAG GAA GGG GAT C	(GA)18	Röder et al., 1998
Xpsp2999	1A	TCCCGCCATGAGTCAATC	TTGGGAGACACATTGGCC	(CAG)n(CAA)n	Devos et al., 1995
Xwmc24	1A	GTGAGCAATTTTGATTATACTG	TACCCTGATGCTGTAATATGTG	(GT)28	Somers et al., 2004
Xgwm577	7B	ATG GCA TAA TTT GGT GAA ATT G	TGT TTC AAG CCC AAC TTC TAT T	(CA)14(TA)6	Röder et al., 1998

*Repeat motif in bread wheat var. Chinese Spring

Table 3 – Changes in the number of alleles, unique alleles and genetic diversity over periods in durum wheat cultivars of Morocco and their comparison to the exotic cultivars.

		Moroccan cultivars before 1990s				Moroccan cultivars of 1990s				Moroccan cultivars of 2000s				Moroccan cultivars before 1990s, 1990s and 2000s			Exotic cultivars			
Locus	Location	Sample size (n)	Number of alleles	Number of unique alleles	Genetic diversity (H)	Sample size (n)	Number of alleles	Number of unique alleles	Genetic diversity (H)	Sample size (n)	Number of alleles	Number of unique alleles	Genetic diversity (H)	Sample size (n)	Number of unique alleles	Genetic diversity (H)	Sample size (n)	Number of alleles	Number of unique alleles	Genetic diversity (H)
Xgwm33	1A	10	3	1	0.460	6	1	0	0	5	3	1	0.640	21	4	0.463	5	1	0	0
Xgwm389	3B	10	4	2	0.580	6	2	0	0.444	5	2	0	0.320	21	4	0.621	5	3	1	0.560
Xgwm146	7B	10	4	3	0.640	6	1	0	0	5	2	1	0.320	21	5	0.463	5	1	0	0
Xgwm397	4A	10	3	1	0.580	6	2	0	0.500	5	2	0	0.480	21	3	0.544	5	2	0	0.480
Xgwm136	1A	10	4	1	0.640	6	2	0	0.500	5	2	0	0.480	21	4	0.671	5	2	1	0.320
Xbarc263	1A	10	2	1	0.180	6	1	0	0	5	1	0	0	21	2	0.091	5	1	0	0
Xgwm130	7A 2B 7B	10	3	1	0.460	6	2	0	0.444	5	3	1	0.640	21	4	0.531	5	2	0	0.320
Xwmc89	4A	10	2	0	0.480	6	2	0	0.500	5	1	0	0	21	2	0.490	5	1	0	0
Xgwm193	6B	10	3	1	0.540	6	2	0	0.500	5	2	0	0.480	21	3	0.526	5	3	1	0.560
Xgwm273	1B	10	2	0	0.420	6	3	1	0.611	5	2	0	0.480	21	3	0.503	5	2	0	0.320
Xpsp2999	1A	10	2	0	0.420	6	1	0	0	5	2	0	0.480	21	2	0.408	5	2	0	0.320
Xwmc24	1A	10	2	0	0.480	6	2	0	0.278	5	1	0	0	21	2	0.363	5	2	0	0.480
Xgwm577	7B	10	5	3	0.720	6	2	1	0.278	5	2	0	0.480	21	6	0.626	5	3	1	0.640
Total			39	14			23	2			25	3			44			25	4	
Mean			3	1.077	0.508		1.769	0.154	0.312		1.923	0.231	0.369		3.384	0.485		1.923	0.308	0.308
Standard deviation (±)			1	1.038	0.136		0.599	0.376	0.234		0.641	0.439	0.230		1.261	0.147		0.760	0.480	0.237

Table 4 – Shared allele genetic distance of 26 cultivars using 13 polymorphic SSR markers.

Cultivar	Amjad	Amria	BelBachir	Cocorit	Icamor	Irden	Jawhar	Karim	Kyperounda	Marouane	Marzak	Medora	Merjana	Nassira	OumRabia	Ourgh	Sarif	Sceptre	Sebou	Selbera	Strongfield	Tarek	Tensift	Tomouh	UC1113	Vitron
Amjad	0.000	0.385	0.615	0.462	0.385	0.462	0.385	0.231	0.692	0.538	0.385	0.538	0.077	0.231	0.615	0.385	0.538	0.538	0.769	0.385	0.615	0.615	0.462	0.462	0.385	0.538
Amria		0.000	0.692	0.538	0.692	0.077	0.462	0.462	0.615	0.308	0.231	0.615	0.385	0.538	0.692	0.615	0.692	0.615	0.538	0.692	0.615	0.692	0.692	0.615	0.538	0.615
BelBachir			0.000	0.462	0.462	0.615	0.385	0.538	0.615	0.538	0.538	0.538	0.538	0.538	0.615	0.538	0.615	0.769	0.615	0.615	0.462	0.154	0.308	0.231	0.462	0.077
Cocorit				0.000	0.538	0.462	0.308	0.308	0.769	0.615	0.385	0.385	0.462	0.462	0.615	0.462	0.538	0.615	0.769	0.615	0.462	0.538	0.538	0.538	0.385	0.462
Icamor					0.000	0.769	0.308	0.308	0.769	0.538	0.692	0.692	0.308	0.154	0.615	0.308	0.462	0.692	0.846	0.615	0.615	0.538	0.538	0.462	0.462	0.462
Irden						0.000	0.538	0.538	0.615	0.385	0.154	0.615	0.462	0.615	0.615	0.692	0.615	0.615	0.538	0.692	0.615	0.615	0.615	0.538	0.615	0.538
Jawhar							0.000	0.154	0.692	0.462	0.462	0.385	0.308	0.154	0.462	0.308	0.385	0.615	0.692	0.692	0.462	0.385	0.538	0.385	0.231	0.308
Karim								0.000	0.846	0.462	0.462	0.385	0.154	0.154	0.615	0.154	0.462	0.462	0.846	0.538	0.462	0.538	0.462	0.538	0.154	0.462
Kyperounda									0.000	0.769	0.615	0.615	0.692	0.615	0.846	0.615	0.538	0.615	0.077	0.462	0.538	0.538	0.692	0.538	0.769	0.615
Marouane										0.000	0.462	0.692	0.462	0.538	0.615	0.462	0.615	0.692	0.692	0.846	0.769	0.615	0.692	0.615	0.538	0.538
Marzak											0.000	0.538	0.385	0.538	0.615	0.615	0.538	0.538	0.615	0.615	0.538	0.538	0.538	0.462	0.538	0.462
Medora												0.000	0.538	0.538	0.615	0.385	0.538	0.231	0.615	0.462	0.154	0.385	0.538	0.538	0.308	0.462
Merjana													0.000	0.154	0.538	0.308	0.462	0.538	0.769	0.385	0.615	0.538	0.462	0.385	0.308	0.462
Nassira														0.000	0.462	0.308	0.462	0.538	0.769	0.538	0.462	0.538	0.538	0.385	0.308	0.462
OumRabia															0.000	0.769	0.615	0.769	0.692	0.692	0.538	0.462	0.769	0.462	0.692	0.538
Ourgh																0.000	0.308	0.462	0.846	0.538	0.462	0.385	0.308	0.538	0.154	0.308
Sarif																	0.000	0.692	0.615	0.692	0.462	0.615	0.538	0.462	0.154	0.462
Sceptre																		0.000	0.615	0.462	0.385	0.615	0.615	0.692	0.692	0.462
Sebou																			0.000	0.538	0.538	0.538	0.692	0.615	0.769	0.615
Selbera																				0.000	0.538	0.538	0.538	0.538	0.538	0.615
Strong field																					0.000	0.308	0.538	0.462	0.385	0.385
Tarek																						0.000	0.308	0.231	0.462	0.077
Tensift																							0.000	0.385	0.462	0.231
Tomouh																								0.000	0.462	0.154
UC1113																									0.000	0.385
Vitron																										0.000

NJ method had grouped the durum wheat cultivars into 6 groups at a genetic distance level of 0.25 (Figure 1), and the exotic durum wheat cultivars Sceptre, Medora and Strong field grouped together and formed a single cluster. Selbera, Sebou, Kyperonda formed a separated cluster. Other two exotic cultivars (Vitron and UC1113-Gpc-B1) were embedded in one cluster where the other Moroccan cultivars are grouped into. PCoA analysis (Figure 2) showed similar results similar to the pattern of NJ method clustering and no clear clustering of varieties to any temporal group was observed.

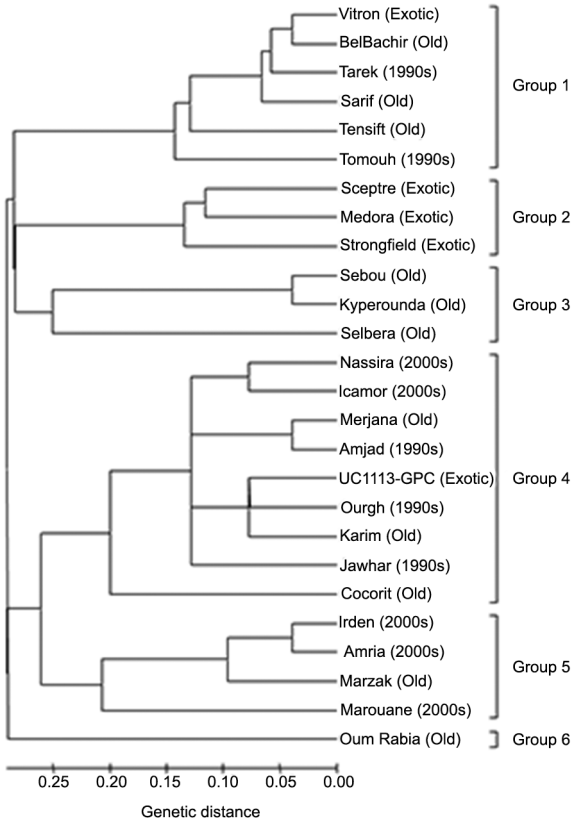

Figure 1 – Dendrogram showing relationships between the 21 Moroccan and 5 exotic cultivars of durum wheat as revealed by the Neighbor-joining method based on shared allele genetic distance. The temporal group or the origin of the cultivar is indicated in parenthesis.

Changes in genetic diversity of durum wheat over time

To analyze the changes in genetic diversity over periods, the 21 cultivars were grouped into three groups ('old cultivars' released before 1990, the 1990s and the 2000s) according to their year of registration. The total number of alleles and unique alleles were highest in cultivars developed before 1990, decreasing in cultivars developed during the 1990s and increasing slightly in cultivars developed during the 2000s (Table 3). The FPt-est clearly showed that decreases in allelic counts in the 1990s and 2000s temporal groups in comparison to the group with 'old cultivars' released before 1990 were significant ($p < 0.05$; Table 5). However, the slight increase in allelic counts in the 2000s temporal group in comparison to the 1990s group was not significant ($p > 0.19$).

Genetic diversity, total alleles and unique alleles were highest in the 'before 1990' temporal group, decreasing in the 1990s, increasing slightly in the 2000's temporal group due to breeding. However, the total genetic diversity of the 1990s and 2000s temporal group was still less than in those cultivars belonging to the 'before 1990' temporal group. AMOVA analysis (Table 6) indicated that most of the molecular variation (91 %) exists among cultivars within temporal groups, with lesser

Table 5 – P-values (based on 50,000 permutations) for FPTest for testing significance of allelic counts between the temporal groups.

	Before 1990	1990s	2000s	Exotic
Before 1990	-	0.0033	0.0401	0.0411
1990s		-	0.1934	0.1935
2000s			-	0.5524
Exotic				-

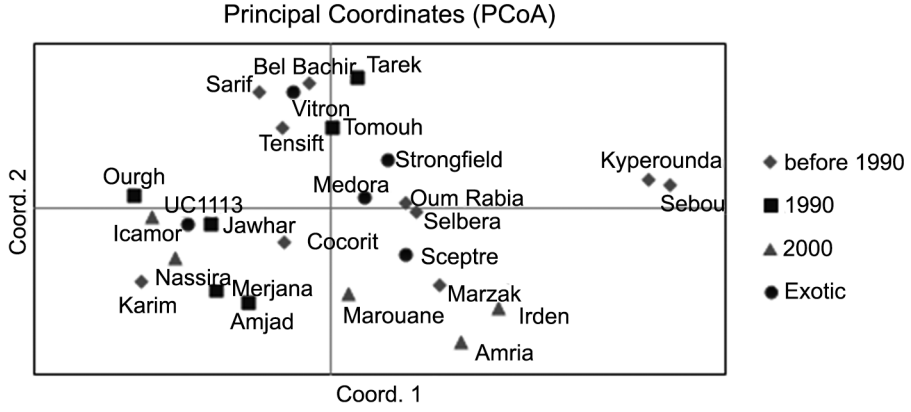

Figure 2 – Principal Coordinate Analysis (PCoA) plot of the Moroccan and exotic durum wheat cultivars based on genetic distance.

Table 6 – Analysis of molecular variance (AMOVA) of durum wheat cultivars from Morocco and exotic origin based on 13 microsatellite marker analysis.

Source	Degrees of freedom (Df)	Sum of squares (SS)	Mean squares (MS)	Est. Var.	%
Among temporal groups	3	58.872	19.624	1.180	9*
Within temporal groups	22	268.667	12.212	12.212	91*
Total	25	327.538		13.392	100

*Significant at $p < 0.05$.

amounts between temporal groups (9 %). Permutation tests (based on 999 permutations) suggest that the overall ΦPT was different from the nil distribution (ΦPT = 0.088, $p < 0.05$). Moreover, there was a reduction in genetic variation between the 'before 1990' and 1990s temporal groups (ΦPT = 0.01, $p < 0.05$). However, the increase in genetic variation between the 1990s and 2000s temporal groups was not significant.

The genetic distances for each pair of temporal groups are summarized in Table 7. The highest genetic distance (0.431) was found between the 2000s and the exotic groups, whereas the lowest (0.272) was observed between the before 1990 and 1990s temporal groups. The cultivars of exotic origin and the before 1990 temporal group clustered separately, whereas the temporal groups from the 1990s and the 2000s grouped together (Figure 3).

Table 7 – Genetic distance between the cultivar registers before 1990 (Old cultivars), the 1990s, 2000s and the exotic durum wheat cultivars.

Groups	Genetic distance			
	Before 1990 (Old)	1990s	2000s	Exotic cultivars
Before 1990 (Old)	0.000	0.272	0.385	0.338
1990s		0.000	0.295	0.308
2000s			0.000	0.431
Exotic cultivars				0.000

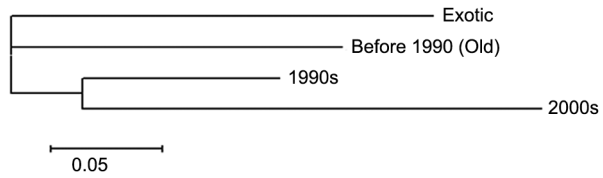

Figure 3 – Dendrogram showing relationships between the three temporal groups and exotic cultivars of durum wheat as revealed by the Neighbor-joining method based on shared allele genetic distance. The scale indicates the genetic distance.

Discussion

The microsatellite analysis has generated information on cultivar relatedness, which is very useful for the breeding program for identification of suitable cultivars to be used as parents in the crossing programs. A set of 13 microsatellites markers was used for deciphering genetic relationships and the characterization of 21 Moroccan durum wheat cultivars and five exotic cultivars. The microsatellites markers used in this study were sufficient to differentiate all the cultivars and can be used as fingerprints for varietal identification. The marker *Xgwm577*, *Xgwm389* and *Xgwm136* were the most informative and showed higher genetic diversity value.

The average number of alleles detected in this study in Moroccan durum wheat cultivars was low (3.38) compared with other recent studies in Tunisia (10.4 alleles; Medini et al., 2005), Syria (7.97 alleles; Achtar et al., 2010), Ethiopia (9.2 alleles; Haile et al., 2013), Iran (5.5 alleles; Mardi et al., 2011) and Italy (4.3 alleles; Figliuolo et al., 2007), indicating a narrow genetic base of Moroccan durum wheat germplasm compared to other countries. Furthermore, our study showed a lower ($p < 0.05$) number of allelic counts in the 1990s and 2000s temporal groups compared to the before 1990 temporal group. The proportional SSR variations within the improved 1990s and 2000s temporal groups were consistently far lower than those within the older cultivars developed before the 1990s. These findings are clearly in line with observations by Fu and Somers, (2011) which demonstrate the association between allelic changes and wheat trait improvements, and are useful for understanding the genetic modification of the wheat genome by long-term wheat breeding. The exotic durum lines currently being deployed in breeding programs will enhance the genetic base of the cultivar in Morocco.

Microsatellites were efficient for studying temporal genetic variation. For instance, studies on temporal changes had reported a reduction in genetic diversity in Italian durum wheat, and breeding processes had been attributed to the reduction compared to landraces (Figliuolo et al., 2007) which contradicted the results of Bulgarian durum wheat (Landjeva et al., 2006), and European winter wheat (Huang et al., 2007) where they reported no declining trends in diversity attributable to the breeding process.

The close genetic relationships observed between a number of the cultivars were explained by the presence of common parents in their pedigree. For instance, the close genetic relationship of durum wheat cultivar Vitron with several of the Moroccan cultivars is also obvious, because many of the Moroccan cultivars are either sister lines of Vitron or have Vitron or its sister lines as one of the parents (Nsarellah et al., 2005). This study is the first to report on genetic characterization of the durum wheat cultivars of Morocco.

In the temporal groups, we detected a decrease in allelic richness in the 1990s and 2000s groups compared with that of the period before 1990 for Moroccan durum wheat cultivars. The slight increase in allelic richness in the 2000s could be explained by the use of varieties introduced and hybridization employing new exotic germoplasm (Jlibene and Nsarellah, 2011). Genetic distance estimates also clearly showed that the durum wheat cultivars of temporal groups before 1990 and the 1990s were closely related compared to genetic distance estimates between the recent temporal group of the 2000s and exotic cultivars, indicating there is an increase in genetic relatedness between the temporal groups which indicates a decrease in genetic diversity. Since there is less of a similarity between temporal groups and exotic cultivars the latter can be employed as parents in a Moroccan breeding program. These findings clearly demonstrate the various natures of the impact of breeding on Moroccan durum wheat cultivars, not only through a reduction in allelic richness but also through a change in genetic relatedness in the released cultivars.

AMOVA showed higher genetic diversity of cultivars within temporal groups (91 %) compared to that between the temporal groups (9 %). A reduction in genetic diversity due to breeding occurring since the 1990s was significant. Similar studies have reported that genetic diversity losses have been observed in recent times attributable to breeding in bread wheat (Christiansen et al., 2002; Reif et al., 2005; Warburton et al., 2006; Huang et al., 2007; Hysing et al., 2008). Even though there was an increase in allelic richness in the recent temporal group (the 2000s) compared to the 1990s, attributable to breeding using exotic germplasm from ICARDA/CIMMYT, overall genetic diversity did not increase ($p > 0.19$). Thus, there is a need to improve further durum wheat productivity and diversity in order to adapt to climate change and emerging pathogens/pests which have been posing real problems in recent years. Exotic durum wheat germplasm are being used as parents in the breeding program for improving productivity and enhancing the genetic diversity of durum wheat on-farm.

Acknowledgements

The authors are grateful to the International Treaty for Plant Genetic Resources for Food and Agriculture/FAO and the ICARDA/Morocco Collaborative Grants Program for the support. We thank Drs. Anil Kumar and Abhishek Rathod, ICRISAT, India for helping to carry out the FPtest. SMU and FH thank CRP-Wheat for the support.

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