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Screening for Drought Tolerance Using Molecular Markers and Phenotypic Diversity in Durum Wheat Genotypes

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Abstract: Wheat is one of the most important staple food crops in the world is adversely affected by drought. Understanding its genetics and genome organization using molecular markers is of great value for plant breeding purposes. The aim of the present study was to assess in formativeness and efficiency of 26 different molecular markers for genetic diversity among 40 durum wheat comprising landraces, old, moderate and improved durum wheat. Phenotypic diversity was evaluated by six traits (yield and its components and time to heading. Significant variation was noted for all parameters. Analysis of principal components contributes determine 74% of the diversity. Cluster analysis was performed to construct dendrogram using Unweighted Pair Group Method with Arithmetic Mean "UPGMA" based on Euclidean distance of genotypes. Five groups were revealed; landraces and old varieties were found to have a characteristic feature of high plant height, moderate yielding and thousand grain weight and reduced days to heading. This indicated that landraces were relatively earlier and relatively taller than the improved varieties. High levels of polymorphism were recorded for SSR markers used in this study. A total of 136 fragments were obtained from the 26 SSR primers and all the bands were polymorphic across all the genotypes screened, most of them were polymorphic. The polymorphism information content (PIC) values ranged from 38 % to 94%, with an average of 74%. These findings provide basis for future efficient use of these molecular markers in the genetic analysis of durum wheat and new strategies could be developed to safeguard and improve our germoplasm for drought tolerance.

Key words: *Durum* wheat · Landraces · Yield · Diversity · PCA · UPGMA · PIC and SSR

INTRODUCTION

Cereals are most important food crops on earth and provide 70 percent of the world's population food. Generally, three quarters of energy and half of essential protein for the human come from grains and cereals. Wheat (*Triticum spp.*) is the second major food crop of the world in its importance next to rice. Durum wheat (*Triticum durum* Desf*)*, is grown mainly in the dry land of the Mediterranean region under stressful and variable environmental conditions. To identify drought tolerant germplasm, the empirical selection approach is employed. For a successful breeding program, the presence of genetic diversity and variability play a vital role. Genetic diversity is essential to meet the diversified goals of plant breeding such as breeding for increasing yield, wider adaptation and desirable quality. Genetic divergence analysis estimates the extent of diversity existed among selected genotypes [1]. The improvement of durum wheat is based on the use of the genetic variability of the local cultivars [2]. These genetic resources contain several important agronomic and resistance genes [3-5].

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In Algeria, several local cultivars are currently threatened to disappear, because they are replaced by the new durum wheat varieties. Thus, to preserve these genetic resources, threatened cultivars should be submitted to reliable conservation and selection program. The use of fluorescently labeled microsatellite markers for genotyping on automated sequencers offers many advantages over analysis using traditional auto-radiographic or silver stained detection techniques [6]. Semi automated methods of SSR genotyping are gradually replacing manual systems in genetics research. These methods facilitate the efficient application of microsatellite markers for high throughput mapping [7], pedigree analysis [8], fingerprinting of accessions [9] and analysis of genetic diversity with more accurate genotyping [10].

In this study the genetic diversity for drought screening was assessed in two ways; analysis of phenotypic diversity permitted the characterization of 40 cultivars and molecular study using 26 microsatellites fluorescently labeled.

MATERIALS AND METHODS

Plant Materials and DNA Isolation: The grains of forty tetraploid wheat genotypes from diverse origin (Table 1) provided from Technical Institute of the Field Crops El khroub Constantine (ITGC) and Wheat NEPAD Project, comprising landraces, these local varieties are commonly cultivated in different location of Algerian condition climate (arid and semi arid) since 1907, differing in annual temperatures and precipitations. Passing yield trials successfully indicates high genetic potential of these cultivars for adaptation to different stresses like drought, heat, cold and salinity, moderate and modern varieties were planted in field at the technical institute crop (ITGC), using lines with 3 m length and 1.2 m width on the basis of a randomized complete block design with three replications. During the growth season the traits including heading time (DH), plant height (PH), number of grains per spike (NGS), thousand grain weight (TGW), number of spikes per square meter (NS/m^2) and grain yield (GY) were measured. For molecular analysis, all wheat cultivars were sown in small plastic pots in a growth chamber providing normal growing conditions at ICARDA center. Total genomic DNA was isolated from fresh leaves by a modification of the method described by Saghai-Maaroof *et al*. [11]. The DNA quality and concentration were estimated using agarose gel (1%), as well as visual comparison with known concentrations of phage lambda DNA.

Table 1: Origin of studied varieties

No	Varieties	Origin	No	Varieties	Origin
1	Bidi17	Algeria	21	HAURANI	Syria/Jordan
2	Col	Italy	22	SAHEL	Algeria (Elkhroub)
3	Can	Italy	23	SENATORE-CAPELLI	Italy
4	Beltagy	ICARDA	24	YAVAROS-79	Cimmyt (Mexico)
5	Bouslem	Algeria	25	Ofonto	Italy
6	Benswif	Egypt	26	Mrf1/Stj2//Gdr2/Mgnl1	ICARDA/Algeria
7	Waha	ICARDA	27	Otb4/3/HFN94N-8/Mrb5//Zna-1	ICARDA/Algeria
8	Oued Znatie	Algeria	28	Oss1/Stj5/5/Bidra1/4/Bezaiz-SHF//SD-19539/Waha/3/Stj/Mrb3	ICARDA/Algeria
9	Hedba3	Algeria	29	F4 13/3/Arthur71/Lahn//Blk2/Lahn/4/Quarmal ICAMOR-TA4-69	ICARDA/Algeria
10	Cirta	Algeria	30	Lahn/Ch12003	ICARDA/Algeria
11	Amar	Algeria	31	Ter-1/3/Stj3//Bcr/Lks4	ICARDA/Algeria
12	Djenah khotifa T	Tunsia	32	Villemur/3/Lahn//Gs/Stk/4/Dra2/Bcr/5/Bcr/Lks4/4/ Bezaiz-SHF//SD-19539/Waha/3/Stj/Mrb3	ICARDA/Algeria
13	line3d	Egypt	33	Adnan-1	ICARDA/Algeria
14	CAPEITI 8	Italy	34	Miki-2	ICARDA/Algeria
15	CHEN 'S'	Cimmyt/Algeria	35	Rahouia	Algeria
16	KORIFLA = SHAM-3	Syria	36	Guemgoum r'kham	Algeria
17	TELL 76	Algeria (Elkhroub)	37	Djenah khotifa	Algeria
18	TASSILI (RABI/FG)	Algeria (Elkhroub)	38	Vitron	Spain
19	COCORIT C 71	Cimmyt (Mexico)	39	Béliouni	Algeria
20	KYPEROUNDA	Cyprus	40	Gta dur	Cimmyt (Mexico)

Table 2: Description of the SSR loci used in this study

Marker	Forwrd primer (5'-> 3')	Reverse primer (5'-> 3')	Len	Lab.	Ps
WMC54	TATTGTGCAATCGCAGCATCTC	TGCGACATTGGCAACCACTTCT	22	NED	142
Wmc63	GTGCTCTGGAAACCTTCTACGA	CAGTAGTTTAGCCTTGGTGTGA	22	VIC	192
WMC78	AGTAAATCCTCCCTTCGGCTTC	AGCTTCTTTGCTAGTCCGTTGC	22	Fam	241
WMC105	AATGTCATGCGTGTAGTAGCCA	AAGCGCACTTAACAGAAGAGGG	22	Fam	192
WMC_150	CATTGATTGAACAGTTGAAGAA	CTCAAAGCAACAGAAAAGTAAA	22	NED	165
WMC_153	ATGAGGACTCGAAGCTTGGC	CTGAGCTTTTGCGCGTTGAC	20	Fam	177
WMC_165	CACACTCGCACGATTTTCCTAT	TCGGTTACACTGGAAGTGGTCT	22	NED	188-193
WMC167	AGTGGTAATGAGGTGAAAGAAG	TCGGTCGTATATGCATGTAAAG	22	VIC	185
WMC168	AACACAAAAGATCCAACGACAC	CAGTATAGAAGGATTTTGAGAG	22	Fam	319
WMC177	AGGGCTCTCTTTAATTCTTGCT	GGTCTATCGTAATCCACCTGTA	22	VIC	184
WMC179	CATGGTGGCCATGAGTGGAGGT	CATGATCTTGCGTGTGCGTAGG	22	VIC	184
WMC235	ACTGTTCCTATCCGTGCACTGG	GAGGCAAAGTTCTGGAGGTCTG	22	VIC	235
WMC307	GTTTGAAGACCAAGCTCCTCCT	ACCATAACCTCTCAAGAACCCA	22	NED	145
WMC322	CGCCCCACTATGCTTTG	CCCAGTCCAGCTAGCCTCC	17	NED	95
WMC445	AGAATAGGTTCTTGGGCCAGTC	GAGATGATCTCCTCCATCAGCA	22	Fam	229
WMS06	CGT ATC ACC TCC TAG CTA AAC TAG	AGC CTT ATC ATG ACC CTA CCT T	22	VIC	207-196
WMS108	ATT AAT ACC TGA GGG AGG TGC	GGT CTC AGG AGC AAG AAC AC	20	Fam	135-137
WMS118	GAT GGT GCC ACT TGA GCA TG	GAT TG TCA AAT GGA ACA CCC	20	Fam	110
WMS135	TGT CAA CAT CGT TTT GAA AAGG	ACA CTG TCA ACC TGG CAA TG	20	VIC	153-176
WMS149	CAT TGT TTT CTG CCT CTA GCC	CTA GCA TCG AAC CTG AAC AAG	21	NED	161
WMS169	ACC ACT GCA GAG AAC ACA TAC G	GTG CTC TGC TCT AAG TGT GGG	22	VIC	220
WMS198	TTG AAC CGG AAG GAG TAC AG	TCA GTT TAT TTT GGG CAT GTG	20	Fam	130
WMS30	ATC TTA GCA TAG AAG GGA GTG GG	TTC TGC ACC CTG GGT GAT TGC	21	VIC	196-205
WMS304	AGG AAA CAG AAA TAT CGC GG	AGG ACT GTG GGG AAT GAA TG	20	VIC	202
WMS375	ATTGGCGACTCTAGCATATACG	GGGATGTCTGTTCCATCTTAGC	22	NED	156-204

Len; length, Lab; labels, Ps; product size

SSR Analysis: For SSR analysis, DNA concentration was adjusted to 50 ng 26 primer pairs (Table 2), were chosen among the publicly available sets catalogued in the Grain Genes database (http://wheat.pw.usda.gov) as *WMC* (*Xwmc*) and as described by Roider *et al*. [12] for *WMS* (*Xgwm*). PCR amplification solution was prepared in a volume of 10 µl using 50 ng genomic DNA, 0.2 mM dNTP, 1.5m M $MgCl_2$, 10 pmol of each primer (forward and reverse), 0.5 U *Taq* polymerase. For multiplexing, sets of 1-3 SSRs with different fluorescent dyes such as blue (FAM), green (VIC), or yellow (NED) for the forward primers have been prepared. The SSR-PCR amplification of genomic DNA was done by incubating the DNA samples at 94°C for 4 min, then 35 cycles comprising 94°C for 1 min, annealing of primer at 58-60°C for 1 min and then extension at 72°C for 1 min. The final extension was carried out at 72°C for 7 min in Applied Biosystems Thermocycler. After PCR amplifications, fragments were electrophoretically separated on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems/HITACHI, Foster city, CA, USA). Before multiplexing markers, each PCR product was optimized for genotyping on ABI 3100. For submission of samples into ABI 3100, 1 µl of this PCR mix was added to 5µl ROX (formamide) containing the Gene scan G350 standard and then heated to 95°C for 5min (denaturation).

Statistical Analysis and Data Scoring: Phenotypic data were analyzed by the General Linear Model (GLM) procedure of the SAS version 9.1 (SAS Institute, 1987, Cary, NC, USA). Agronomical traits were used in multivariate analysis with the major goals to distinguish between varieties and to determine the main characters that allow differentiation between the varieties and geographical origin. The distance between individus, was calculated with similarity coefficient of Manhattan "complete linkage", than regroupment was performed with method of Unweighted Pair Groups Method of Analysis (UPGMA). These analyses were carried out using the DARwin 5.0.148 software program available at (http://darwin.cirad.fr/darwin/Home.php).

Molecular Data Analysis: After extracting microsatellite data from the ABI 3100 sequencer, they were analyzed for allele calls with Gene Mapper software version 3.7 (*Applied Biosystems*). Allelic variation and polymorphism information content (PIC) was analyzed using Power marker 3.25 (http://statgen.ncsu.edu/powermarker/index.html).

RESULTS AND DISCUSSION

Phenotypic Evaluation: The means, standard deviation, covariance coefficient and ranges of six measured agronomic traits for 40 studied genotypes are presented in (Table 3). The studied varieties showed high significant differences in all six traits at P<0.01 (Table 4). Coefficient of variance (CV %) for all studied traits are shown in Table 3. High CV values were shown for NS/m^2 (74.40%) and NGS (79.46 %) indicating high variation in the studied genotypes for these traits (Table 3). Significant positive correlations (P<0.01) were observed between NGS, TGW and GY (Table 5). The strongest positive correlation was between TGW and NGS (r = 0.84) and between GY and TGW (r = 0.81) confirming the findings of Koksal [13] that thousand grain weight increased the yield directly. Gashaw *et al.* [14] showed also that thousand grain weight had significant positive correlation with grain yield and suggested that these traits could be used as a direct criteria for improving yield of durum wheat (Table 5). The principal components analysis (PCA) explained 74 % of the total variation (Fig. 1). The first axis explained 54% of the total variability and the most associated traits were days to heading, number of spikes/m^2, number of grains per spike, weight of thousand grains and yield. The first dimension can be named as the yield potential and drought tolerance. The second PCA axis explained 20% and was mostly related to plant height, weight of thousand grains and yield. T h is axis e valuate the reproductive vigor PH, yield and compounds of yield (number of grains per main s pike Thousand grain weight, number of spikes per meter square) and phenological traits (days to heading).

Table 3: Mean performance values for the studied traits

Variable	Minimum	Maximum	Mean	SD	CV%
DH	99.00	130.00	107.74	5.17	7.09
PH	51.00	126.00	93.19	2.56	18.38
NS/m^2	49.00	433.00	192.46	2.86	74.40
NGS	16.72	438.00	227.35	4.16	79.46
TGW	20.00	46.30	38.26	1.47	15.72
GY	18.00	46.60	34.41	1.78	21.05

Table 4: Analysis of variance for the studied traits

	ANOVA			
Traits	d.f	SS	MS	F
DH	39	6397	164**	23.8
PH	39	34764	891**	465.5
NS /m^2	39	2037034	52232**	11431
N G/S	39	1244980	31923**	15662.5
TGW	39	4263.2	109,3**	191.5
GY	39	6184.3	158,6**	204.2

** Significant at 0.01 level of probability.

Table 5: Relationship between phenotypic traits for all studied genotypes

Traits	DH	PH	NS/m2	NGS	TGW	GY
DH	1	0,226	0.005	0.180	0.242	0.154
PH		1	0.302	0.256	0.111	0.142
NS/m^2			1	0.687	0.542	0.711
NGS				1	0.845	0.738
TGW					1	0.809
GY						1

*, **: Significant at 0.05 and 0.01 levels of probability, respectively

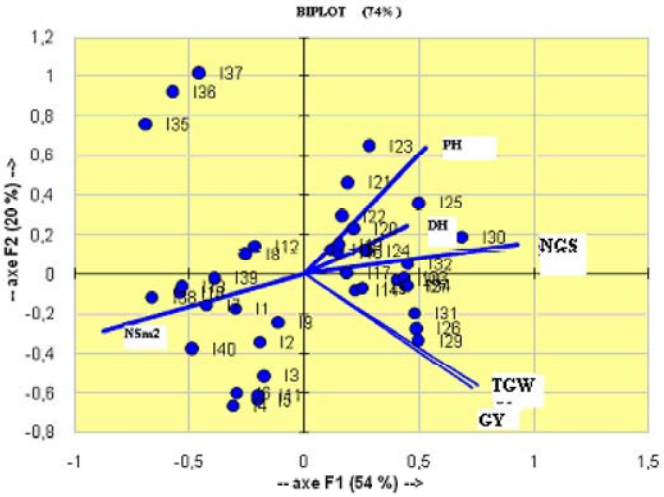

Fig. 1: Distribution of the 40 Durum wheat accessions for the agronomical and phenological Traits in the plan of axes 1 and 2.

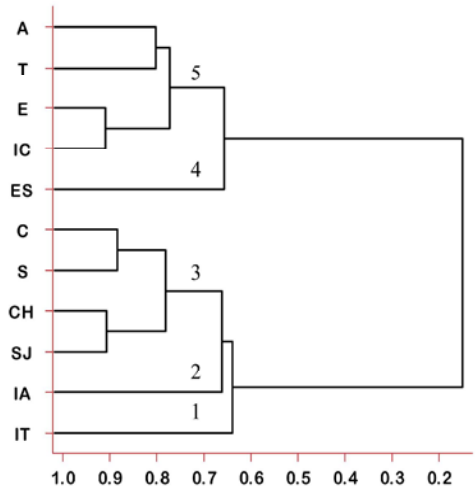

Fig. 2: Cluster mean analysis. Euclidean genetic matrices of 40 genotypes were made using UPGMA based on Euclidean distance of genotypes geographic origin.
A. Algeria, IT. Italy, IC. Icarda, T. Tunisie, E. Egypt, C. Cimmyt, S.syrie, CH.chypre, SJ. Syrie jordanie, IA. Icarda/algeria and ES. Spain.

Cluster, completing PCA analysis and according to genotypes geographical origin, based on their phenotypic traits, five groups were revealed. Also, the genetic distance, calculated of this cluster revealed that these five distances were significantly distant and lie between the ranges of 9.1%, observed between CIMMYT and SYRIA to 47.05% obtained between Spain and ICARDA/Algeria (Fig. 2). The first group includes genotypes from Algeria, Tunisia, Egypt and ICARDA, this group was found to have a characteristic feature of high plant height, moderate yielding and thousand grain weight and reduced days to heading. This indicated that landraces were relatively earlier and taller than the improved varieties. This is a typical feature of landraces, which excel in capacity to support panicle growth by large stem reserve mobilization [15-17]. The second group includes Spain varieties characterized by reduced plant height and days to heading and low yielding. The third group includes Cimmyt, Syria, Cyprus and Syria/Jordan genotypes characterized by low NGS, while the fourth group consists of ICARDA/Algeria genotypes characterized by high number of grains per spike, thousand grain weight and moderate days to heading. The last one contains Italy genotypes with long days to heading (Fig. 2).

Genetic Diversity and SSR Markers Characteristics: Data derived from these experiments were analyzed to evaluate the usefulness of the microsatellites for genetic diversity of the 40-durum wheat varieties. The genetic diversity was measured by the polymorphic information content (PIC). According to Vaiman *et al.* [18], loci polymorphism can be considered high, medium or low if PIC>0.5, 0.5>PIC>0.25 and PIC<0.25, respectively. A total of 136 fragments were obtained from the 26 SSR primers, the majority of bands were polymorphic across all genotypes screened. The majority of primers used in this study generated polymorphic profiles with variable and significant genetic diversity. Data in Table 6 represents the allelic frequency and all SSR estimated parameters of diversity, so all studied genotypes showed genetic diversity, presenting high heterozygosis attainted 0.9750

Table 6: Characteristics of the SSR markers and their number of alleles, allele frequency, availability, heterozygosity and PIC values calculated for 40 durum wheat genotypes

	Major.			Gene		
Marker	Allele Frequency	Allele no	Availability	Diversity	Heterozygosity	PIC
wmc63	0.4500	9.0000	1.0000	0.6972	0.9750	0.6516
WMC165_1	0.2750	9.0000	1.0000	0.8075	0.6500	0.7816
WMC165_2	0.5000	9.0000	0.9500	0.7147	0.0000	0.6960
WMC445	0.7500	5.0000	0.7000	0.4133	0.0000	0.3858
WMC150_1	0.4211	11.0000	0.9500	0.7116	0.8947	0.6698
WMC150_2	0.5000	11.0000	0.9000	0.6894	0.1111	0.6575
WMC177_1	0.1375	24.0000	1.0000	0.9400	0.1750	0.9369
WMC177_2	0.2778	10.0000	0.9000	0.8422	0.0556	0.8247
WMC78_1	0.1500	22.0000	0.5000	0.9350	0.5000	0.9315
WMS06	0.3333	8.0000	0.3000	0.8090	0.0833	0.7866
WMC105_1	0.2353	18.0000	0.8500	0.8676	0.1471	0.8558
WMC105_2	0.2778	11.0000	0.4500	0.8549	0.1111	0.8409
WMS149	0.3974	16.0000	0.9750	0.7748	0.4103	0.7519
WMC235	0.3333	4.0000	0.1500	0.7222	0.0000	0.6713
WMS304	0.1795	23.0000	0.9750	0.9191	0.3846	0.9140
WMS198	0.4306	22.0000	0.9000	0.7797	0.2500	0.7661
WMS375	0.3289	14.0000	0.9500	0.7715	0.1316	0.7411
WMS135	0.6216	11.0000	0.9250	0.5961	0.0000	0.5823
WMC168	0.5556	4.0000	0.2250	0.6173	0.0000	0.5688
WMC322	0.3158	14.0000	0.9500	0.8148	0.8684	0.7934
WMC54_1	0.7167	9.0000	0.7500	0.4706	0.1333	0.4541
WMC54_2	0.4342	10.0000	0.9500	0.7510	0.1316	0.7258
WMC153_1	0.7167	5.0000	0.7500	0.4494	0.0333	0.4113
WMC153_2	0.5294	9.0000	0.8500	0.6799	0.0000	0.6566
WMC167	0.2188	12.0000	0.4000	0.8652	0.1875	0.8520
WMS169	0.2000	20.0000	1.0000	0.9056	0.9000	0.8988
WMS108	0.3194	18.0000	0.9000	0.8148	0.3889	0.7958
WMS30_1	0.1290	23.0000	0.7750	0.9251	0.7097	0.9203
WMS30_2	0.2727	12.0000	0.2750	0.8719	0.3636	0.8614
WMC307	0.4545	4.0000	0.5500	0.6570	0.0909	0.5934
WMC179	0.2375	17.0000	1.0000	0.8794	0.6500	0.8691
WMS118	0.2237	17.0000	0.9500	0.8736	0.8684	0.8623
Mean	0.3726	12.8438	0.7719	0.7632	0.3189	0.7409 □ 0.0255

for *wmc*63 primer. The locus *wms*135 showed 11 homozygous alleles (Table 6). The heterozygosis mean for the 26 SSR primer pairs was about 0.318. The components of genetic diversity of each marker obtained with an average data availability of 76% are shown in Table 6. In the present study, SSR markers were able to discriminate between the 40 wheat genotypes studied. The nine genomic microsatellite primers *wmc*177, *wmc*78, *wms*304, *wms*30, *wmc*105, *wmc*179, *wms*118, *wms*149 and *wms*375 were sufficient to differentiate all of the wheat genotypes since they generated a high number of alleles with high PIC values. The primers *WMC*54 and *WMC*153 were less polymorphic with PIC values 0.45 and 0.41, respectively (Table 6). Among the 26 SSR primer pairs used in this study, 24 SSR primers were polymorphic. In total, 136 alleles were detected for SSR primers, with an average of 13 alleles per locus. The lowest number of alleles was presented by *wmc*235 and *wmc*168 (4 alleles), while the highest one was of *wmc*177 (24 alleles). Summarized data for the SSR loci and their PIC values are presented in Table 6. The PIC value, a reflection of allelic diversity and frequency among the durum wheat genotypes analyzed, were generally high for all tested SSR loci. PIC values ranged from 38 % to 94%, with an average of 74%, which 20 SSR loci revealed PIC values higher than 0.70. Among these; *wmc*177, *wmc*78, *wms*304, *wms*30, *wmc*105, *wmc*179, *wms*118, *wms*149 and *wms*375 are noteworthy due to their relatively high polymorphism (24, 22, 23, 23, 18, 17, 17, 16 and 14 alleles) and high PIC values (94%, 94%, 91%, 92%, 86%, 87%, 86%, 75% and 74%), respectively.

The PIC can be looked as the measurement of usefulness of each marker in distinguishing one individual from another. The PIC values and rare alleles are proved to be useful information in genetic diversity analysis of genotypes. The polymorphism of SSR loci detected in this study was consistent with data obtained in some previous studies [19, 20]. The simple sequence repeats (SSRs) represent the most suitable marker system in wheat [21] and have been successfully used to characterize genetic diversity in advanced wheat breeding materials [22].

This study provides a detailed analysis and quantification of genetic diversity in wheat genotypes. Data also supported the finding that microsatellites can be effectively used for studying genetic diversity in durum wheat. Some of these markers will be used in MAS in future wheat breeding programmes. The alleles revealed by markers showed a high degree of polymorphism, this suggested that the genotypes selected for this study harbored enough genetic divergence. The markers showed an average PIC values of which confirm that SSR markers used in this study were highly informative because PIC values higher than 0.50 indicate high polymorphism. Markers with PIC values of 0.5 or higher are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of marker at specific locus [23].

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