Association Analysis of Genotypic and Phenotypic Traits Using SSR Marker in Durum Wheat

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Abstract

In durum wheat, marker-trait associations were studied for six agronomic traits using a set of 25 simple sequence repeat (SSR) markers , with a set of 40 wheat genotypes including drought, semi-tolerant and non-drought tolerant genotypes. According to the factorial discriminate analysis (FDA) for phenotype traits and to the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster, the 40 genotypes, were classified into five distinct groups. The first two components of FDA results for phenotype traits, explained 74% of total variation. The results show that landraces were earlier and relatively taller than the improved varieties. A total of 14 SSR markers showed significant associations with studied agronomic traits on chromosomes of durum wheat. Some markers (*wmc*54 (3B), *wms*118 (5B) and *wmc*165 (3A), showed significant associations with several traits and were associated with Number of grains per main spike (NGS), Number of spike per meter square (NS/m^2)and throusand kernel weight (TKW) as well as with grain yield (GY) and its components. In total, 136 alleles were obtained with a mean of 13 alleles/locus. The average PIC value was 0.74 suggesting a high degree of genetic diversity. The analysis using the Mantel matrix correspondence test, revealed a positive and highly significant correlation (r=0.6096; p<0.01, 1000 random permutations) between the agro-morphological traits and the microsatellite marker-derived similarity matrices. Furthermore, the molecular variance analysis (AMOVA) showed that the proportion of variance explained by within and among geographical groups diversity was 83 % and 17%, respectively. Thus, our study showed significant variation in morphological traits and microsatellite DNA polymorphisms among wheat varieties.

KEYWORDS: durum wheat, FDA, association analysis, SSR marker, PIC, AMOVA.

Introduction

Wheat production is largely influenced by climate variability and weather extremes. The increased frequency of drought and other abiotic stresses negatively affects crop yields (Araus *et al*., 2002 ; Howden *et al.* 2007, Slafer and Araus, 2007; Khan *and al* 2011, Lakew and al ., 2012). However, drought resistance in *durum wheat* is a complex trait that is governed by quantitative trait loci (QTL) (Tuberosa and Salvi, 2006 ; Lang and al , 2010 ;Livaja and al., 2011). Selection efficiency of drought-resistant traits could be enhanced with a better understanding of its genetic control.

Molecular markers associated with a quantitative trait in plants are traditionally identified using a population derived from a biparental cross. This requires substantial time for the development of a recombinant inbred line populations. The complementary method of association mapping has been proven to be useful and powerful for genetic dissection of complex traits. Historically originating from human genetics (Templeton and al., 1995), association mapping is emerging as a novel tool in plant genomics (Myles and al., 2009) and utilizes diverse natural plant populations in detecting the correlations between genes/markers and traits of interest (Yu *and al.,* 2006) . The QTL mapping and analysis provides unprecedented opportunities to identify and locate chromosome regions controlling adaptive traits such as time to heading, plant height (Wang *and al*. 2010; Wu *an*d al, 2010 ;Zhang *and al.*, 2011) and yield (Quarrie and al*.,* 2006).

The advantages of population-based association study (Ibrokhim and al*.,* 2008), utilizing a sample of individuals from the germplasm collections or a natural population over traditional QTL-mapping in biparental crosses primarily, are due to (1) availability of broader genetic variations with wider background for marker-trait correlations (i.e., many alleles evaluated simultaneously), (2) likelihood for a higher resolution mapping because of the utilization of majority recombination events from a large number of meiosis throughout the germplasm development history, (3) possibility of exploiting historically measured trait data for association, and (4) no need for the development of expensive and time-consuming biparental populations (Kraakman and al, 2004; Hansen and al., 2001). Recently, AM has been used in various crops to detect molecular markers associated with a variety of complex traits. Simple sequence repeat (SSR) markers were significantly associated with various agronomic traits in rice, with kernel size and milling quality of wheat (*Triticum aestivum* L.) (Breseghello and al., 2006), flowering time of perennial ryegrass (Skot and al*.,* 2007), dynamic development of plant height in common wheat (Zhang and al, 2011) and salinity tolerance of barley (*Hordeum vulgare* L.) (Eleuch *and al.,* 2008), and drought tolerance in barley (Varsheny and *al*. 2012 and Lakew *and al*. ,2012).

The objectives of the present work were to characterize a collection of durum wheat genotypes (landraces and improved varieties) with a number of simple-sequence repeat (SSR) microsatellites and to identify associations between specific alleles and variation in the expression of traits important for rainfed agriculture. Such associations would be essential prerequisites for marker-assisted selection (MAS) of traits in a breeding programme for improved drought resistance.

Material and methods

Plant material and DNA isolation

For this study 40 durum wheat genotypes were used (**Table 1**). The cultivars are characterized by contrasting agricultural productivity, collected from various sources, obtained from the Technical institute of the Field Crops El khroub CONSTANTINE (Algeria) (TIFC) and wheat New Partnership for Africa's Development (NEPAD) project, comprising 13 old local varieties/populations. These varieties have been commonly cultivated in different locations of Algerian climate (arid and semi arid) , differing in annual temperatures and precipitations. Passing those yield trials successfully indicates the high genetic potential of these varieties for adaptation to different stresses like drought, heat, cold and salinity, these old varieties /population are more cultivated and the most appreciated (Ducellier, 1930) such as Beliouni, Rahouia, Bidi and Hedba3 . The collection comprises also; five 'founder genotypes' widely used as parents in breeding programmers throughout the Mediterranean Basin and at International Centers (CIMMYT and ICARDA) like Waha;, Gta dur, Djenah khotifa, , Vitron and Sahel (ITGC , 1995), modern varieties released between 1980 and 1996 and nine new varieties and elite lines were provided by the International Center for Agricultural Research in the Dry Areas (ICARDA). All varieties were grown in a randomized block design with three replications at the experimental field of TIFC.

The annual rainfall and its monthly distribution differed from year to year at TIFC station, Algeria . The total annual rainfall was 363 mm. In general, rainfall was lower than the long-term average (487 mm) in 25 preceding years, rainfall in the months of March and April was low and much less than the crop requirements. This low rainfall and its poor distribution affected crop performance; it subjected the crop to severe drought stress, particularly during the grain filling period. However, the months of March and April were warmer, drier, with more evaporative demand than in the last 25 years.

Phenotypic data of these varieties, were measured for plant height PH (cm), yield and its compounds (Number of grains per main spike (NGS), thousand kernel weight (g) TKW, Number of spike per meter square (NS/m^2) and Grain yield (q/ha) GY) and phenological traits (Days to heading (days) DH.

For molecular analysis, the total genomic DNA was isolated from fresh leaf material (at ICARDA), by a modification of the method described by (Saghai- Maroof *and al.,* 1984). DNA quality and concentration were estimated by agarose gel (1%) electrophoresis, as well as by visual comparison with known concentrations of phage lambda DNA.

Table.1 ***Origin, pedigrees and release information of studied varieties***

N°	Varieties	Origin	Pedigrees	Release information
1	Bidi17	Algeria	Old local landrace	1930
2	Colosseo	Italy	CRESO/MEXA	1990
3	Cannizzo	Italy	Advanced/improved cultivar	1998
4	Beltagy3	ICARDA	ICD97-0396-T-1AP-AP-5AP-0AP-16AP-AP	2008
5	Bouslem	Algeria	HEIDER/MARTE//HUEVO DE ORO ICD86-0414-ABL-OTR-4AP-OTR-14AP-OTR	2007
6	Benswif	EGYPT	Corm"S"/Rufo"S"	1995
7	waha	ICARDA	PELICANO/RUFF//GAVIOTA/ROLETTE;	1979
8	Oued Znatie	Algeria	old local landrace	1936
9	Hedba3	Algeria	Polonicum x ZB	1921
10	Cirta	Algeria	Hedba3/GDO VZ 619	2000
11	Amar6	Algeria		2010
12	Djenah khotifa T	Tunisia	LV-NORTH-AFRICA[39]; Breeding line	1955
13	line3d	Egypt	MSWD-3	
14	CAPEITI 8	Italy	Eiti-6/SENATORE-CAPPELLI;EITI-8/SENATORE-CAPPELLI;	1955
15	CHEN 'S'	Cimmyt/Algeria	Shwa'S'/Bit'S'CD 26406-3B-2Y-9Y-OM-3YOB	1989-1990
16	KORIFLA = SHAM -3	Syria	DURUM-DWARF-S-15/CRANE//GEIER,TR.DR[1281];	1926
17	TELL 76	Algeria (Elkhroub)	CRANE(SIB)/F3-TUN//(SIB)ANHINGA/3/(SIB)FLAMINGO	1976
18	TASSILI (RABI/FG)	Algeria (Elkhroub)	GDO VZ 469/Jo"S"X 61.130-Lds= Stk"S"CM 470-1M-3Y- 0KB	1985
19	COCORIT C 71	CIMMYT (Mexico)	RAE/4*TC60//STW63/3/AA'S'=CISNE D.27617	1971
20	KYPEROUNDA	Cyprus	landrace	1927
21	HAURANI	Syria/Jordan	landraces (Breeding line)	1988
22	SAHEL = Cit's'XPg'S'-AA'S'/Ruff X T.Dic.Ver-Gll'S'=Loon'S'	Algeria (Elkhroub)	CMN 528-C-1Y-1M-0Y	1985
23	SENATORE-CAPELLI	Italy	(S)JENAH-RHETIFAH;(S)JEAN-RHETIFAH	1915
24	YAVAROS-79	CIMMYT	JORI-	1979

		(Mexico)	69(SIB)/(SIB)ANHINGA//FLAMINGO,MEX;	
25	Ofonto	Italy	ADAMELLO/APPULO	1992
26	Mrf1/Stj2//Gdr2/Mgnl1	ICARDA/Algeria	ICD01-0946-C-AP-13AP-TR	2010
27	Otb4/3/HFN94N-8/Mrb5//Zna-1	ICARDA/Algeria	ICD00-1095-T-AP-12AP-AP-6AP-TR	2008
28	Oss1/Stj5/5/Bidra1/4/Bezaiz-SHF//SD-19539/Waha/3/Stj/Mrb3	ICARDA/Algeria	ICD00-0393-T-9AP-AP-12AP-AP	2008
29	F4 13/3/Arthur71/Lahn//Blk 2/Lahn/4/Quarmal ICAMOR-TA4-69	ICARDA/Algeria	ICD00-0334-T-2AP-0AP-13AP-AP-4AP-0AP	2008
30	Lahn/Ch12003	ICARDA/Algeria	ICD00-0393-T-9AP-AP-13AP-AP	2011
31	Ter-1/3/Stj3//Bcr/Lks4	ICARDA/Algeria	ICD1036-T-0AP-9AP-AP-5AP-AP	2011
32	Villemur/3/Lahn//Gs/Stk/4/Dra2/Bcr/5/Bcr/Lks4/4/Bezaiz-SHF//SD-19539/Waha/3/Stj/Mrb3	ICARDA/Algeria	ICD00-0388-T-AP-12AP-AP-1AP-TR	2010
33	Adnan-1	ICARDA/Algeria	ICD97-0494-T-13AP-AP-5AP-0AP-1AP-AP	2008
34	Miki-2	ICARDA/Algeria	ICD94-0994-CABL-10AP-0AP-1AP-0AP	2008
35	Rahouia	Algeria	old local landrace	1960
36	Guemgoum r'kham	Algeria	old local landrace	1960
37	Djenah khotifa	Algeria	Local landrace selection from North Africa	1955
38	Vitron	Spain	TURCHIA-77/3/JORI(SIB)/(SIB)ANHINGA//(SIB)FLAMINGO;	1986
39	Béliouni	Algeria	old local landrace	1958
40	Gta dur	Cimmyt (Mexico)	Gaviota /durum	2007

SSR Analysis

Twenty five primer pairs (**Table.2**), were chosen among the publicly available sets catalogued in the *GrainGenes* database (http://wheat.pw.usda.gov) for *wmc* (*Xwmc*) and as described by Röder et al. (1998) for *wms* (Xgwm). PCR amplification was prepared in a volume of 10µL using 50 ng genomic DNA, 0.2mM dNTP, 1.5mM $MgCl_2$, 10 pmol of each primer (forward and reverse) and 0.5U *Taq* polymerase. For multiplexing, sets of 1–3 SSRs with different fluorescent dyes such as blue (*FAM*) (5-

carboxy fluorescéine), green (*VIC*) (tétrachloro_fluorescéine), or yellow 2'-chloro-5'-fluoro-7',8'-fused phenyl-1,4-dichloro-
6-carboxyfluorescein (*NED*) for the forward primers has 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, 1µL of this PCR mix was added to 5µL ROX (formamide) containing the Genescan G350 standard and then heated to 95 °C for 5 min.

Table. 2 Description of the SSR loci used in this study len: length, lab: labels, ps: product size, loc: location

Marker	Forward Primer (5' -> 3')	Reverse Primer (5' -> 3')	len	lab	ps (pb)	loc
WMC54	TATTGTGCAATCGCAGCATCTC	TGCGACATTGGCAACCACTTCT	22	NED	**142**	3B
wmc63	GTGCTCTGGAAACCTTCTACGA	CAGTAGTTTAGCCTTGGTGTGA	22	VIC	**192**	2A
WMC78	AGTAAATCCTCCCTTCGGCTTC	AGCTTCTTTGCTAGTCCGTTGC	22	Fam	**241**	3B
WMC105	AATGTCATGCGTGTAGTAGCCA	AAGCGCACTTAACAGAAGAGGG	22	Fam	**192**	6B
WMC_150	CATTGATTGAACAGTTGAAGAA	CTCAAAGCAACAGAAAAGTAAA	22	NED	**165**	2A
WMC_153	ATGAGGACTCGAAGCTTGGC	CTGAGCTTTTGCGCGTTGAC	20	Fam	**177**	3A
WMC_165	CACACTCGCACGATTTTCCTAT	TCGGTTACACTGGAAGTGGTCT	22	NED	**188-193**	3A
WMC167	AGTGGTAATGAGGTGAAAGAAG	TCGGTCGTATATGCATGTAAAG	22	VIC	**185**	2B
WMC168	AACACAAAAGATCCAACGACAC	CAGTATAGAAGGATTTTGAGAG	22	Fam	**319**	7A
WMC177	AGGGCTCTCTTTAATTCTTGCT	GGTCTATCGTAATCCACCTGTA	22	VIC	**184**	2A
WMC179	CATGGTGGCCATGAGTGGAGGT	CATGATCTTGCGTGTGCGTAGG	22	VIC	**184**	2A
WMC235	ACTGTTCCTATCCGTGCACTGG	GAGGCAAAGTTCTGGAGGTCTG	22	VIC	**235**	5B
WMC307	GTTTGAAGACCAAGCTCCTCCT	ACCATAACCTCTCAAGAACCCA	22	NED	**145**	3B
WMC322	CGCCCCACTATGCTTTG	CCCAGTCCAGCTAGCCTCC	17	NED	**95**	3A, 3B
WMC445	AGAATAGGTTCTTGGGCCAGTC	GAGATGATCTCCTCCATCAGCA	22	Fam	**229**	5A

WMS06	CGT ATC ACC TCC TAG CTA AAC TAG	AGC CTT ATC ATG ACC CTA CCT T	22	VIC	**207 - 196**	4B
WMS108	ATT AAT ACC TGA GGG AGG TGC	GGT CTC AGG AGC AAG AAC AC	20	Fam	**135- 137**	3B
WMS118	GAT GGT GCC ACT TGA GCA TG	GAT TG TCA AAT GGA ACA CCC	20	Fam	**110**	5B
WMS135	TGT CAA CAT CGT TTT GAA AAG G	ACA CTG TCA ACC TGG CAA TG	20	VIC	**153 -176**	1A
WMS149	CAT TGT TTT CTG CCT CTA GCC	CTA GCA TCG AAC CTG AAC AAG	21	NED	**161**	4B
WMS169	ACC ACT GCA GAG AAC ACA TAC G	GTG CTC TGC TCT AAG TGT GGG	22	VIC	**220**	6A
WMS198	TTG AAC CGG AAG GAG TAC AG	TCA GTT TAT TTT GGG CAT GTG	20	Fam	**130**	4A
WMS30	ATC TTA GCA TAG AAG GGA GTG GG	TTC TGC ACC CTG GGT GAT TGC	21	VIC	**196-205**	3A
WMS304	AGG AAA CAG AAA TAT CGC GG	AGG ACT GTG GGG AAT GAA TG	20	VIC	**202**	5A
WMS375	ATTGGCGACTCTAGCATATACG	GGGATGTCTGTTCCATCTTAGC	22	NED	**156-204**	4B

Statistical analysis and data scoring

Phenotypic data were analyzed by the MIXED procedure of the SAS version 9.1 (SAS Institute, 2000, 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 based on their geographical origin by Factorial discriminate analysis (FDA) using Genetix version 4.04 (Belkhir *and al,* 1999). The distance between individuals, was calculated with similarity coefficient of "Manhattan" complete linkage", than regroupment was performed with method of *UPGMA* (Unweighted Pair Groups Method of Analysis), These analyses were carried out using the DARwin 5.0.148 software program available at (http://darwin.cirad.fr/darwin/Home.php).

Molecular data

After extracting microsatellite data from the ABI 3100 sequencer, they were analyzed for allele calls with GeneMapper software version 3.7 (Applied Biosystems). The genetic structure of the population was analyzed using Structure 2.1http//pritch.bsd.uchicage.edu/structure.html). The optimal group number (*K*, from 2 to 8 tested in this study) of the population was estimated with reference to the description of (Evanno *and al.,* 2005) . When an inflexion emerges in the LnP(D) curve, the corresponding *K* value is adopted as the optimal group number. The *Q* values were calculated to serve as covariates in the association analysis that was carried out using Tassel 2.0 (http://www2.maizegenetics.net/) adopting a general linear model (GLM) (Bradbury et al. 2004). Allelic variation and polymorphism information content (PIC) were analyzed using Powermarker 3.25 (http://statgen.ncsu.edu/powermarker/index.html), and cluster trees were drawn using DARwin 5.0 (http://darwin.software.informer.com) by Jaccard distance (Jaccard, 1908). The relationships between the similarity matrix based on phenotytipic and

genetic similarity matrix obtained with microsatellite was analyzed according to Mantel (1967) using the NTSYSpc ver. 2.01 program (Sneath and Sokal,(1973).

An analysis of molecular variance (AMOVA) (Excoffier *and al.,* 1992) across all studied material using Genestat software , AMOVA was used to partition the total SSR variation into within geographical regions and among geographical regions components (Excoffier and al.,, 1992).

Results and discussion

Phenotypic traits

High significant difference was identified between genotypes for all phenotypic traits (**Table 3**) According to genotypes geographical origin, the genetic distance between CIMMYT (Gta dur, Yavros-79, Cocorit C 71and Chen 'S') and Syria (Korifla), ICARDA(Waha and Beltagy) Varieties was the lowest 0.091, however the distance between Spain and ICARDA/Algeria was the highest (47.052) (**Fig. 1**).The UPGMA dendrogram clearly shows the relationships among 11 geographical regions.

Table.3 Mean phenotypic values of all studied traits

Variable	Minimum	Mean	CE	CV%	SL
DH	99	107.74	5.17	7.09	***
PH	51	93.19	2.56	18.38	***
NS/m2	49	192.46	2.86	74.4	***
NGS	16.72	227.35	4.16	79.46	***
TGW	20	38.26	1.47	15.72	***
GY	18	34.41	1.78	21.05	***

CV: variation coefficient;

CE: critcal etendue

Sl: significant Level

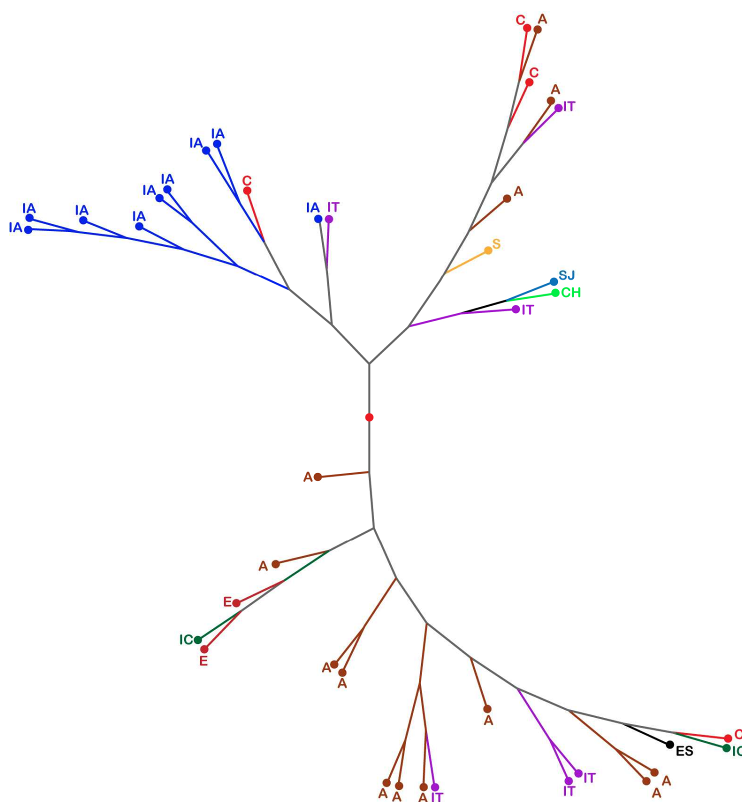

Figure 1. Unweighted Pair Group Method using Arithmetic Means (UPGMA).based on Euclidean distance of genotypes origin geographic
A. Algeria, **IT.** Italy, **IC**. ICARDA , **T**. Tunisia, **E**. Egypt, **C**. CIMMYT **S.** Syria **CH**.Cyprus **, SJ**. Syria Jordan, **IA** . ICARDA/Algeria and **ES**. Spain

The relationships observed in the UPGMA analysis were mirrored in the Factor Discriminate Analysis (FDA), so the first two components of FDA for regions, explained 74% of total variation in the estimates of genetic similarity, which was highly informative. Altogether, five distinct groups were revealed by the first two principal components (**Fig. 2**) ; the first group, including genotypes from Algeria, Tunisia, Egypt and ICARDA was found to have a characteristic feature of high plant height, moderate yields and thousand grain weight with reduced days to heading. This indicated that landraces were relatively earlier and relatively taller than the improved cultivars. These are typical features of landraces, which excel in capacity to support panicle growth by large stem reserve mobilisation. (Masood *et al.*, 2005; Ayed and al., ., 2010).; The second group including varieties from Spain were characterised by reduced PH and DH and low GYs ; the third group, including varieties from CIMMYT, ICARDA, Cyprus, Jordan were characterised by low NSS; the fourth group consisted of varieties from ICARDA and Algeria were charaterised by high NGS, TKW, and medium DH, the last one had genotypes from Italy with long DH. (**Fig. 2**)

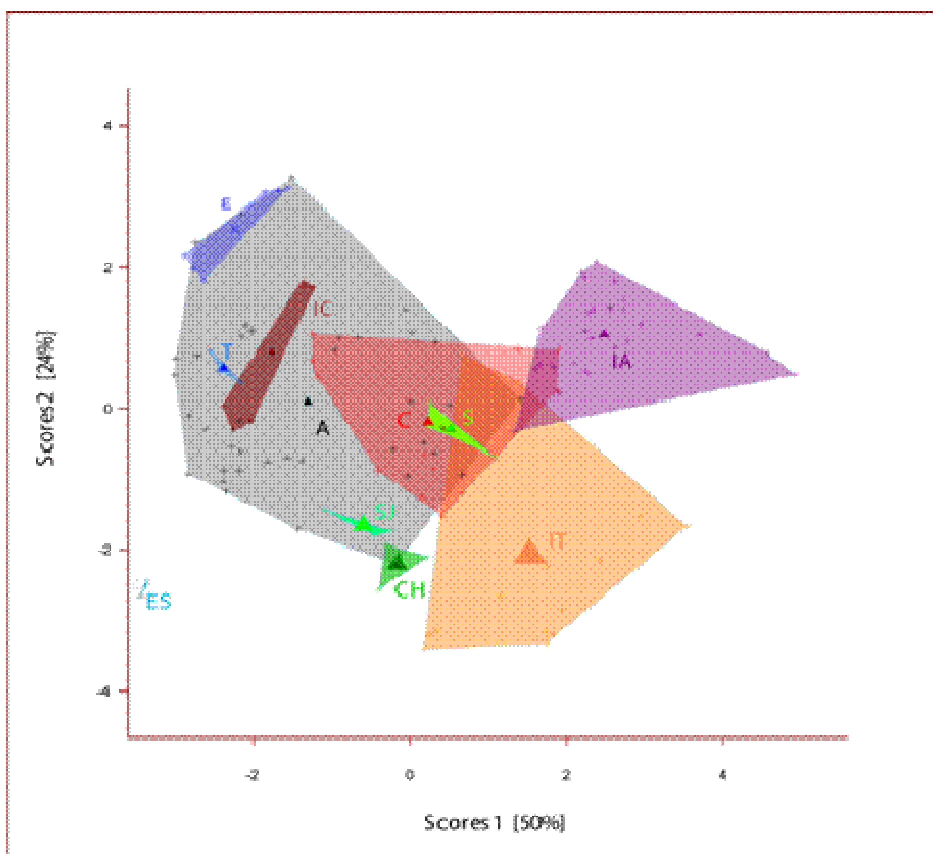

Figure 2. Factorial Discriminant Analysis (FDA) of phenotypic traits and distribution of the studied durum wheat varieties for their geographical origin in the plan of axes 1 and 2 (50% and 24 %)..

A. Algeria, **IT**. Italy, **IC**. ICARDA , **T.** Tunisia, **E.** Egypt, **C.** CIMMYT **S.** Syria CH.Cyprus , **SJ.** Syria Jordan, **IA** . ICARDA/Algeria and **ES.** Spain

Population structure

Using the 25 SSR loci, six groups were most reasonably identified for the population of 40 varieties. This division of the population was supported by statistical probability and could ensure the accuracy of association analysis with a minimum of false association. Based on the population structure at *K*=6, association analysis was conducted, which could avoid the influence of artificial division (Gupta and al., 2005).

Marker-trait association

The TASSEL (Trait Analysis by aSSociation, Evolution, and Linkage) program was used to detect associations between markers and phenotypic data by using a general linear model. A total of 14 *SSR* markers showed significant associations with six agronomic traits on chromosomes of durum wheat (**Table.4**). Most of the significantly associated markers with the examined traits were mapped on chromosome 4A and 3B. The results indicate that these region of chromosome 4A and 3B are important for drought tolerance in durum wheat.

These SSR markers, significantly associated with phenotypic traits in GLM, explained between 32.46% and 99.46% phenotypic variation.(Maccaferri and al., 2008 and

Diab and al., 2008) , reported that a genomic region on chromosome 3B and 4A were associated with drought resistance traits such as stomatal resistance, leaf canopy, time to heading, yield components and harvest index in wheat genotypes

Table.4 Agronomic traits associated with SSR markers

Trait	Marker	P	R^2 %
DH	*WMS149*	5.078^{ns}	88.83
	WMS6	0.001***	96.84
	WMC445	0.05*	32.46
NGS	*WMS30_1*	0.002***	98.87
	WMS375	0.006**	57.73
	WMC165_2	0.011**	46.23
	WMS118	0.02*	78.1
	WMC54_2	0.05*	44.83
	WMS169	0.054*	82.46
NS/m2	*WMC165_2*	0.005**	49.88
	WMC54_2	0.013**	51.79
	WMS149	0.04*	60.98
TKW	*WMS375*	0.005**	58.64
	WMC165_2	0.01**	46.45
	WMS149	0.02*	64.41
	WMC153_2	0.03*	45.71
	WMS30_1	0.03*	96.58
	WMS118	0.03*	76.72
	WMC54_1	0.03*	49.8
	WMS304	0.04*	76.57
GY	*WMS149*	0.008**	68.94
	WMS30_2	0.021*	99.46
	WMC177_2	0.03*	49.74
PH	*WMC177_2*	0.03*	49.08

* ,**, *** significant at 0,05, 0.01, 0,1 levels of

probability, respectively.

Days to Heading

Time of flowering is a major trait of a crop adaptation to the environment. Developing short-duration varieties has been an effective strategy for minimizing yield loss from terminal drought, as early maturity helps the crop to avoid the period of environmental stress (Kumar and Abbo*,* 2001; Araus *and al.,* 2003)

Two markers showed significant association with DH. The *wms6* (p<0.01) located on chromosome 4B accounted for 96.84 % of the phenotypic variation. In addition, the marker *wmc445* (p<0.05), located on chromosome 5A accounted for 32.46% of variation (**Table 4**) . These markers were detected in local varieties like O.Zenati (allele 198pb) for *wms6* and for *wmc445* in Bouslem (allele 260pb), these landraces were earlier for DH in Mediterranean environments. Our results are in agreement with the previous studies of (Kato *and al.*,2000 ; Huang *and al.*, 2003; Li *and al.,* 2007) and (Araus *and al* ., 2003. Diab *and al.*, 2008; Maccaferri *and al.*, 2008); for the QTLs locations of DH and GY on chromosomes 3B and 5A.).

Morphological traits

For PH, one significantly associated marker was detected. The marker *Xwmc177* (P<0.05), located on chromosome 2A accounted for 49.08% of the phenotypic variation, this marker was detected in genotypes Amar, (253 pb), Benisweif, Beltagy, Cannesseo and Cirta characterized by less PH. Our results are in agreement with the findings of (Yao and al., 2009) who mapped one QTL for PH on chromosome 2A. In addition to this (Quarrie and al, 2005; 2006, Marza and al., 2006 ; Maccaferri and al, 2008, Rebetzke and al., 2008 , Zhang *and al.*, 2008 and Zhang and al ., 2011) detected PH-QTLs on other chromosomes: 3A, 3B, 4B , 5A, 6A, 7A, and 7B.

Yield and its components

For this trait, three significantly associated markers were detected with yield *(WMS149* (p<0.01), *WMS30_2*) (p<0.05) and *WMC177_2* (p<0.05)), localized respectively on chromosomes 4B, 3A and 2A, their contributions were 68.94%, 99.46% and 49.74% of the phenotypic variation (**Table 4**). All QTLs identified in this study were mapped earlier by Merza and al., (2006) who identified the location of QTLs on chromosomes 1A, 1B, 2B, 3B, 4B, 5B, 7A, and 7B. Mathews *and al.,* (2008) mapped eight Yield QTLs on chromosome 4B; and these locations were also identified by others (Bo¨rner *and al.*, 2002; Peng *and al.*, 2003; Huang and al., 2004; McCartney *and al.*, 2005), on chromosome 2AL.

The NGS was found to be significantly associated with six markers: (*WMS30* (p<0.001) (129 , 208pb), *WMS*375(140pb) (p<0.01), *WMC*165 p<0.01 (257pb), *WMS118* (198pb) (p<0.05) , *WMC54* (153pb) (p<0.05) and *WMS*169 (p<0.05) , their contribution ranged from 44,83% to 98.87% of the phenotypic variation, located on chromosomes 3A, 4B, 3A, 5B, 3B and 6A respectively. These loci were positively correlated with increased NGS.

Three markers showed significant association for NS/m^2. These markers were located on chromosomes 3AS, 3A and 4B. Their contributions ranged from 49.88% to60.98% of the total NSm^2 variation. For TKW eight putative markers *WMS375* (140pb)

($p<0.01$), *WMC165* (257pb) ($p<0.01$) , *WMS*149 (145pb) ($p<0.05$) , *WMC*153 (175pb) ($p<0.05$) , *WMS*30 (208pb) ($p<0.05$) , WMS118 ($p<0.05$) (199pb), WMC54(94pb) ($p<0.05$) and *WMS*304 (209pb) ($p<0.05$) , which are located on chromosomes 4B, 3A, 4B, 3A, 3A,5B, 3B and 5A, respectively. Their contributions ranged from 45.71% to 96.58% and its alleles with a positive effect were positively correlated with high TKW. This finding confirms the QTLs location detected by (Sun *and al.*, 2009 and Yao and al., 2009).

Co-located markers

Since yield and morphological traits are correlated, association-trait analysis was also conducted to detect co-located markers. Out of 14 significantly associated SSR markers detected, 50% of these markers were contributing for more than one trait. Some markers were found to be linked to different traits on chromosomes 2A (*Xwmc177)*, 3B (wms 30) and (*Xwmc* 54), 4B (*WMS*149), (*WMS* 375) , 3A (*Xwmc 165)* , 5B (*WMS 118*) Some markers showed significant effects on several traits; as an example, *Xwmc* 54 (3B), (*WMS 118*) 5B, (*Xwmc* 65) 3A were associated with NGS, NS/m2 and TKW as well as with GY and its components.

Source of alleles for drought tolerance

Drought tolerance genes are located throughout the genome and are genotype dependent. In this study, we found that for TKW eight significantly associated markers were detected in tolerant varieties to drought and show high TKW in rain fed conditions. However, some of the markers were also detected in drought sensitive varieties like (*WMS* 177) 2A for PH. (Börner and al., 2002) and (Huang and al., 2003) found similar results for other traits of interest. These findings further confirm that drought tolerance is a quantitative trait and that apparently sensitive varieties may contain alleles for tolerance, which may not be found in the tolerant varieties.

Genetic diversity of durum wheat

The genetic diversity was measured by the polymorphic information content (PIC). According to Vaiman et al. (1994), loci polymorphism can be considered high, medium or low if PIC>0.5, 0.5>PIC>0.25 and PIC<0.25. In 40 genotypes, 23 SSR primer pairs have a PIC>0.5, with an average of 0.74 (Table.5), indicating that the majority of SSR markers could contribute substantial information to the genetics and breeding of durum wheat. A total of 136 fragments were obtained from the 26 SSR primers and the majority of the bands were polymorphic across all the genotypes screened. Primers used in this study, generated polymorphic profiles with variable and significant genetic diversity. **Table 5** presents the allelic frequency and all SSR estimated parameters of diversity, the majority of studied genotypes show genetic diversity, presenting high heterozygosity attained 0.9750 for *WMC 6*3 (**Table 5**). The mean heterozygosity for the 26 SSR primer pairs was about 0.318.. In the present study, SSR markers were able to discriminate between the 40 wheat genotypes studied. The nine genomic microsatellite primers *WMC177, WMC78 , WMS304 , WMS30, WMC105, WMC179, WMS118, WMS149* and *WMS375***,** were sufficient to differentiate all of the wheat genotypes since they generated a high number of alleles with high PIC values. The primers *WMC54, WMC153*, were, as expected, less polymorphic with PIC values 0.45 and 0.41 respectively (**Table 5**). The polymorphism of SSR loci detected in this study was consistent with data obtained in some previous studies (Royand al., 2006 ; Taranto *and al.,* 2010).

Table. 5 Characteristics of the SSR markers used and their number of alleles, allele frequency heterozygosity and PIC values calculated for a set of 40 durum wheat genotypes

Marker	*Major.* Allele.Frquency	*Allele no*	*Availability*	*Gene* Diversity	*Heterozygosity*	*PIC*
wmc63	0,45	9,00	1,00	0,70	0,98	0,65
WMC165_1	0,28	9,00	1,00	0,81	0,65	0,78
WMC165_2	0,50	9,00	0,95	0,71	0,00	0,70
WMC445	0,75	5,00	0,70	0,41	0,00	0,39
WMC150_1	0,42	11,00	0,95	0,71	0,89	0,67
WMC150_2	0,50	11,00	0,90	0,69	0,11	0,66
WMC177_1	0,14	24,00	1,00	0,94	0,18	0,94
WMC177_2	0,28	10,00	0,90	0,84	0,06	0,82
WMC78_1	0,15	22,00	0,50	0,94	0,50	0,93
WMS06	0,33	8,00	0,30	0,81	0,08	0,79
WMC105_1	0,24	18,00	0,85	0,87	0,15	0,86
WMC105_2	0,28	11,00	0,45	0,85	0,11	0,84
WMS149	0,40	16,00	0,98	0,77	0,41	0,75
WMC235	0,33	4,00	0,15	0,72	0,00	0,67
WMS304	0,18	23,00	0,98	0,92	0,38	0,91
WMS198	0,43	22,00	0,90	0,78	0,25	0,77
WMS375	0,33	14,00	0,95	0,77	0,13	0,74
WMS135	0,62	11,00	0,93	0,60	0,00	0,58
WMC168	0,56	4,00	0,23	0,62	0,00	0,57

WMC322	0,32	14,00	0,95	0,81	0,87	0,79
WMC54_1	0,72	9,00	0,75	0,47	0,13	0,45
WMC54_2	0,43	10,00	0,95	0,75	0,13	0,73
WMC153_1	0,72	5,00	0,75	0,45	0,03	0,41
WMC153_2	0,53	9,00	0,85	0,68	0,00	0,66
WMC167	0,22	12,00	0,40	0,87	0,19	0,85
WMS169	0,20	20,00	1,00	0,91	0,90	0,90
WMS108	0,32	18,00	0,90	0,81	0,39	0,80
WMS30_1	0,13	23,00	0,78	0,93	0,71	0,92
WMS30_2	0,27	12,00	0,28	0,87	0,36	0,86
WMC307	0,45	4,00	0,55	0,66	0,09	0,59
WMC179	0,24	17,00	1,00	0,88	0,65	0,87
WMS118	0,22	17,00	0,95	0,87	0,87	0,86
Mean	0,37	12,84	0,77	0,76	0,32	0,74

Mantel test

The cluster analysis (**Fig. 3**) clearly shows a relationship for microsatellite profiles of the local varieties and the exotic one to their respective geographical origin. In order to study the extent of agreement between dendrograms derived from phenotypic traits and microsatellite markers, the respective distance matrices were compared using the (Mantel, 1967) matrix correspondence test. The analysis revealed a positive and highly significant correlation (r=0.6096; p<0.01, 1000 random permutations) between the agro-morphological and microsatellite marker-derived similarity matrices.

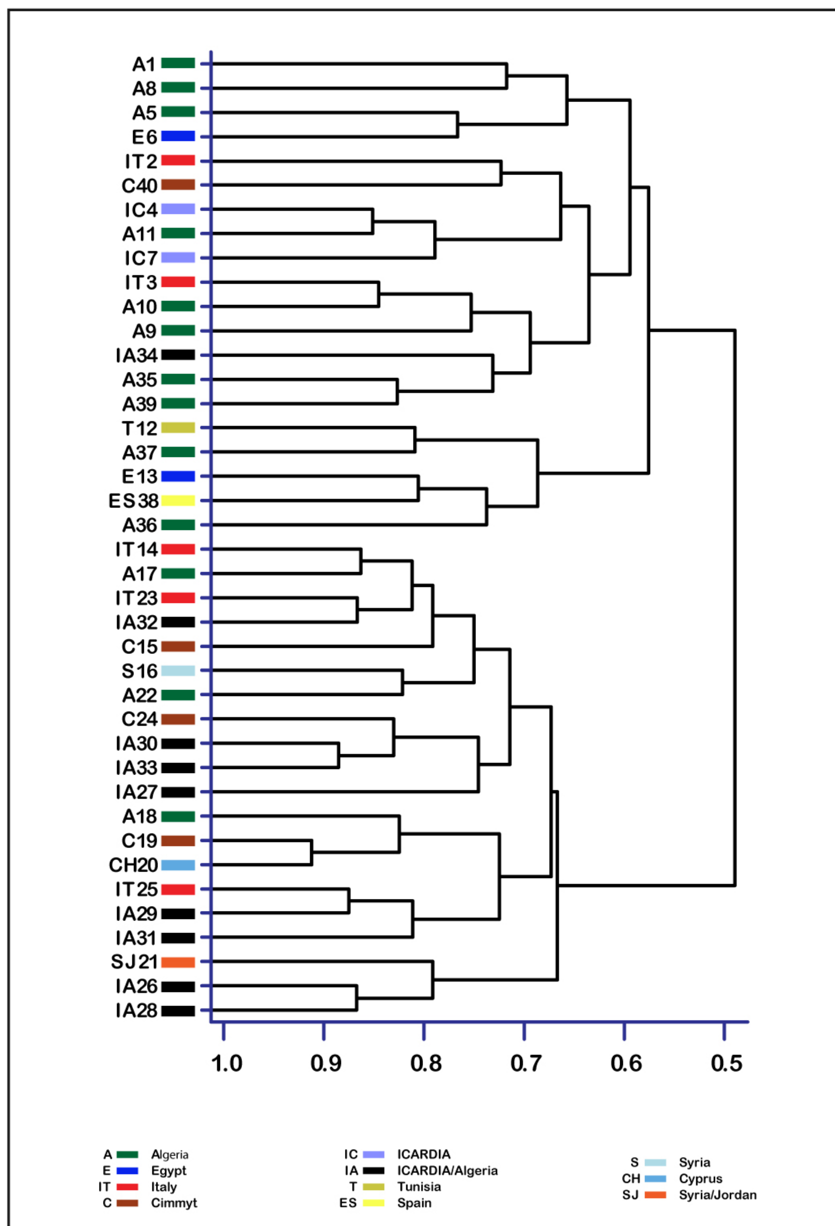

Figure 3. Hierarchical Cluster Analysis of the germplasm collection based on their geographical origin and microsatellite profiles.

A. Algeria, **IT**. Italy, **IC**. ICARDA , **T.** Tunisia, **E.** Egypt, **C.** CIMMYT **S.** Syria CH.Cyprus , **SJ.** Syria Jordan, **IA** . ICARDA/Algeria and **ES.** Spain

AMOVA variation

The AMOVA analysis results revealed 83% and 17% of variation presented among and within geographical groups (ICARDA, CIMMYT, Algeria, Italy, Tunisia, Egypt, Syria, Cyprus, Syria/Jordan, ICARDA/Algeria and Spain) respectively, which revealed large variation within regions. This indicated that there is a significant ($p<0.01$) geographical pattern of diversity distribution of *durum wheat.*

Conclusion

It can be concluded that the sensitive varieties may contain some tolerance alleles, that when combined with alleles from tolerant varieties can result in an increased level of tolerance. In summary, our data showed significant variation in morphological traits and microsatellite DNA polymorphisms among wheat varieties. The *wms* and *wmc* data can be used in selecting diverse varieties in breeding programs for improvement of traits needed for adaptation to various stress conditions. The obtained results provide an excellent opportunity to develop stress tolerant crops. These results may be helpful in wheat breeding programs aimed at improving drought tolerance.

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