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Microsatellite analysis reveals a progressive widening of the genetic basis in the elite durum wheat germplasm

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Abstract It has been argued that the level of genetic diversity in the modern durum wheat (*Triticum turgidum* L. var. *durum*) elite germplasm may have declined due to the high selection pressure applied in breeding programs. In this study, 58 accessions covering a wide spectrum of genetic diversity of the cultivated durum wheat gene pool were characterized with 70 microsatellite loci (or simple sequence repeats, SSRs). On average, SSRs detected 5.6 different allelic variants per locus, with a mean diversity index (DI) equal to 0.56, thus revealing a diversity content comparable to those previously observed with SSRs in other small-grain cereal gene pools. The mean genetic similarity value was equal to 0.44. A highly diagnostic SSR set has been identified. A high variation in allele size was detected among SSR loci, suggesting a different suitability of these loci for estimating genetic diversity. The B genome was characterized by an overall polymorphism significantly higher than that of the A genome. Genetic diversity is organised in well-distinct sub-groups identified by the corresponding foundation-genotypes. A large portion (92.7%) of the molecular variation detected within the group of 45 modern cvs was accounted for by SSR alleles tracing back to ten foundation-genotypes; among those, the most recent CIMMYT-derived founders were genetically distant from the old Mediterranean ones. On the other hand, rare alleles were abundant, suggesting that a large number of genetic introgressions contributed to the foundation of the well-diversified germplasm herein considered. The profiles of recently released varieties indicate that the level of genetic diversity present in the modern durum wheat germplasm has actually increased over time.

Keywords Durum wheat · SSRs · Genetic distance · Germplasm · Fingerprinting · *Triticum turgidum* L. var. *durum*

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Introduction

It has been suggested that the elite germplasm of the major crops, especially self-pollinating cereals, has experienced an overall reduction of its genetic basis as a result of high selection pressures, recurrent use of the adapted elite germplasm and the adoption of breeding schemes not favouring genetic recombination (Allard 1996; Rosegrant et al. 1997; Hoisington et al. 1999). However, the debate as to the extent and the consequences of this reduction in diversity is still open (Le Buanec 1999; Smale et al. 2001) and more detailed phenotypic and molecular data are required (Donini et al. 2000b). On the other hand, genetic gains in yield potential, adaptation and tolerance to multiple biotic and abiotic stresses need to be ensured to sustain the predicted future increase in food demand (Pfeiffer et al. 2000).

Durum wheat (*Triticum turgidum* L. var. *durum*, 2n = 4*x* = 28; AABB genomes) is an important crop, mainly used for human consumption. Recently, this cereal has been the object of renewed interest, because of its valuable production and adaptation to low rainfall and semiarid environments. More than half of the durum acreage lies in the Mediterranean basin, mainly Italy, Spain, France, Greece and the West Asian and North African (WANA) countries, where through history this cereal has received special attention as an important commodity (Royo et al. 2000). The genetic structure of the durum germplasm cultivated in the Mediterranean basin varies largely, encompassing traditional landraces, varieties directly obtained from the local materials, more recent and successful CIMMYT-derived cvs as well as modern varieties characterized by high yield potential,

wide adaptability (also in terms of yield stability) and technological quality (Nachit et al. 1998; Pfeiffer et al. 2000).

Among the Mediterranean countries, Italy has the longest tradition in durum wheat breeding (Bozzini et al. 1998) and its germplasm can be considered as one of the richest and most valuable. There are indications that in the past decades the genetic basis of the durum wheat elite germplasm may have been eroded (Pecetti and Annicchiarico 1998), mainly due to the limited differentiation of the end-use, the high quality level required by customers and the uniformity of the plant ideotype pursued by breeders, coupled with the diffusion of a relatively small number of outstanding genotypes with proven adaptability and yield potential. Autrique et al. (1996) observed that a limited number of ancestral lines contributed largely to the development of the modern durum wheat materials and that the molecular fingerprints of a few ancestors accounted for the majority of the molecular diversity detected in the cultivated gene pool. Molecular markers provide a powerful tool to analyse genetic relationships. In cereals, extensive information is available for rice (Ishii and McCouch 2000; Temnykh et al. 2001), maize (Mumm and Dudley 1994; Smith et al. 1997; Lu and Bernardo 2001) and barley (Melchinger et al. 1994). Genetic diversity in the *Triticeae* has been explored using a range of molecular markers (reviewed in Gupta et al. 1999; Koebner et al. 2001). Among small-grain cereals, barley and bread wheat adapted germplasm has been investigated in detail with amplified fragment length polymorphisms (AFLPs; Barrett and Kidwell 1998) and simple sequence repeat loci (SSRs; Prasad et al. 2000; Russell et al. 2000); only recently molecular diversity surveys have focused on durum wheat (Eujail et al. 2002; Soleimani et al. 2002).

The evaluation of genetic changes occurred over time and the degree of persistence of chromosome regions tracing to known ancestors can be conveniently monitored with the use of single-locus, mapped molecular markers, particularly SSRs. Retrospective analyses of the genetic diversity, integrated by phenotypic and/or pedigree data, have been carried out in barley (Russell et al. 2000) and bread wheat (Donini et al. 2000a; Manifesto et al. 2001) but not in durum wheat. A detailed knowledge, at the molecular level, of the genetic structure and variability of improved populations and elite materials is useful for a more effective planning and management of breeding programmes (Pfeiffer 2000). The preservation in the adapted germplasm of successful allelic combinations obtained over repeated breeding cycles is also an important target when exploiting exotic genetic variability. Consequently, information as to the abundance and polymorphism level of molecular markers in the adapted germplasm of each crop is valuable when addressing breeding issues.

In wheat, microsatellite markers are becoming the markers of choice, due to the level of polymorphism shown even in closely related varieties (Plaschke et al. 1995; Donini et al. 1998) and the uniform distribution in the wheat genome (Röder et al. 1998), while they have also proved reliable and suitable for multiplexing. The richness in allelic variants shown by dinucleotide repeats is particularly valuable for estimating relatedness based on molecular data, since the upward bias of genetic similarities versus co-ancestries will be substantially reduced (Lynch 1988). In the allopolyploid wheats, in most cases SSRs behave as genome-specific markers (single-locus markers). Several SSRs have been isolated and mapped in wheat and more markers are being developed *in silico* and through international collaborative efforts (Eujail et al. 2002; Kantety et al. 2002; see the GrainGenes web site at http://www.graingenes.org). Due to the transferability of SSRs, the results of molecular studies carried out on the A and B genomes in bread wheat can be fully exploited in durum wheat. SSRs retain a highly successful amplification rate and conservation of map position across the *Triticeae* maps (Korzun et al. 1999; Peng et al. 2000; Nachit et al. 2001).

Herein, we report on the use of highly informative dinucleotide SSRs covering all of the durum wheat chromosomes to investigate the genetic diversity present in elite durum wheat accessions. Additionally, important ancestors of modern varieties and check cvs have been included to ascertain to what degree breeding practices may have led to a reduction in the genetic basis of modern, elite durum wheats.

Materials and methods

Plant material

The 58 durum wheat accessions listed in Table 1 were selected in order to represent the most relevant durum germplasm in Italy and other Mediterranean countries. The 58 accessions included: (1) 34 Italian cvs recently released or widely grown in the last four decades, characterized either by a broad adaptability across environments or adapted to specific agro-geographical areas; (2) five French, four U.S. and two recently released CIMMYT cvs, all of them well-adapted to Mediterranean environments; (3) ten cvs (seven obtained in Italy and three from the CIMMYT breeding program) released from 1915 to 1984, hereafter called "founders", and representing important milestones in the durum breeding programmes for the Mediterranean areas; and (4) two Italian (Russello SG7 and Saragolla) accessions and one Tunisian (Inrat 69) accession selected to represent the allelic variants present in the native landraces mostly grown in the centre of the Mediterranean basin before the advent of modern breeding. The pedigree, country of origin, year of release and seed source for each accession are reported in Table 1. Pedigree information was obtained from either published (Brajcich et al. 1986) or web-based pedigree databases (e.g. http://www.ars-grin.gov) and from the annual publications of the Italian durum wheat national list (available on request).

Microsatellite analysis

Genomic DNA was extracted from young leaves of 20 plantlets according to Saghai-Maroof et al. (1984). Initially, eight distantly related accessions were screened for polymorphism using 120 *Xgwm* SSR loci isolated and mapped in bread wheat by Röder et al. (1998). These loci were chosen based on the available map information and on the results of previous studies in wheat (Plaschke et al. 1995; Röder et al. 1995; Fahima et al. 1998). The

Table 1 List of durum wheat accessions used in the SSR analysis. Year of release, country of origin, pedigree, breeder and seed source of each accession are reported. Bold denotes founder-genotypes

Genotype	Year of release	Country of origin	Registered pedigree	Breeder	Seed source
Adamello	1985	Italy	Valforte/Turkish line	Ist. Sper. Cerealicoltura	a
Appio	1982	Italy	Cappelli//Gaviota/Yuma	FederConsorzi	b
Appulo	1973	Italy	Cappelli/Grifoni//Capeiti 8	Consorzio Riforma Fondiaria Bari	a
Arcangelo	1983	Italy	Creso/Appulo	Consemalmo Bari	a
Arcobaleno	1995	Italy-Spain	Chen/Altar 84	Semillas Battle	a
Bronte	1996	Italy	Berillo/Latino	Ist. Sper. Cerealicoltura	b
Capeiti 8	1940	Italy	Cappelli/Eiti	Stazione Granic. Sicilia	c
Cappelli	1915	Italy	Strampelli' selection from Jennah Khetifa	Strampelli	a
Ciccio	1996	Italy	F6 Appulo/Valnova//Valforte/Patrizio	Eurogen	d
Cirillo	1992	Italy	Jucci/Polesine//Creso/Montanari	Maliani Genetica	d
Colosseo	1995	Italy	Mutante di Mexa/Creso	Eurogen	d
Creso	1974	Italy	Yt 54-N10-B/2* Cp 63//3*TC 60/3/Cp B 14	ENEA	d
Duilio	1984	Italy	Cappelli//Anhinga/Flamingo	FederConsorzi	b
Flaminio	1998	Italy	Latino/Cappelli	SIS	b
Flavio	1992	Italy	Latino/Cappelli	SIS	b
Fortore	1995	Italy	Capeiti 8/Valforte	Ist. Sper. Cerealicoltura	a
Gargano	1997	Italy	Trinakria/Valforte//Valnova/Appulo	Ist. Sper. Cerealicoltura	a
Grazia	1985	Italy	M 6800127/Valselva	Maliani Genetica	d
Ionio	1995	Italy	Lira/Vic	SPB	d
Iride	1996	Italy	Altar 84/Ares (= Ionio)	SPB	d
Italo	1993	Italy	Complex cross between Italian and Turkish genotypes	Mosconi	d
Karel	1980	Italy	Mex.198/Maristella	Centro Reg. Cagliari	e
L35	–	Italy	Altar 84/Ares (= Ionio)	SPB	d
Latino	1982	Italy	Cappelli/Aningha//*T. turgidum*	FederConsorzi	b
Lira B 45	1985	Italy	Mandon/FD 1104	SPB	d
Messapia	1982	Italy	Mex./Crane "S"//Tito	Ist. Migl. Bari	d
Ofanto	1990	Italy	Appulo/Adamello	Ist. Sper. Cerealicoltura	d
Platani	1995	Italy	Valnova/Capeiti	Stazione Granic. Sicilia	e
Plinio	1988	Italy	Line D50/Trigo Candeal	FederConsorzi	b
San Carlo	1996	Italy	Grazia/Degamit	Maliani Genetica	d
Simeto	1988	Italy	Capeiti 8/Valnova	Stazione Granic. Sicilia	d
Solex	1995	Italy	Creso/Valgerardo	Giordani	d
Svevo	1996	Italy	CIMMYT's Selection/Zenit	SPB	d
Trinakria	1970	Italy	B 14/Capeiti 8	Ist. Agronomia Palermo	d
Valbelice	1992	Italy	0111/BC 5	Ist. Agronomia Palermo	a
Valforte	1980	Italy	Yt54-N10-B/2*BY//LD390 II 14587 /3/Cappelli*2/ Yuma	Ist. Sper. Cerealicoltura	e
Valnova	1975	Italy	Yt54-N10-B/2*BY//LD390 II 14587/3/Cp/4/Cp/ Yuma	Ist. Sper. Cerealicoltura	a
Varano	1997	Italy	Capeiti 8/Creso//Creso/3/Valf./Trinakria	Ist. Sper. Cerealicoltura	d
Vitromax	1996	Italy-Spain	Turchia77/3/Jori/Anhinga//Flamingo	Semillas Battle	a
Vitron	1987	Italy-Spain	Turchia77/3/Jori/Anhinga//Flamingo	Semillas Battle	a
Zenit	1992	Italy	Valriccardo/Vic	SPB	d
Russello SG7	–	Italy	Selection from the Italian landraces "Russie"	–	c
Saragolla	–	Italy	Selection from the Italian landraces "Saragolle"	–	c
WB881	n.a.	U.S.A.	Complex cross of Ward-Wells-Cando-Waskana-Mexicali 75	WPB	d
Kronos	n.a.	U.S.A.	APB MSFRS POP Sel (D03-21)	APB	d
Produra	1980	U.S.A.	TME/2*TC60//ZB/Wells/3/TC60/2*BYE// Tecur125E /2*TC60	Northrup King	e
Colorado	1995	U.S.A.	P 92/932-2	Pioneer Hi Bred	a
Durfort	1996	France	Selected from the REVA population	Verneuil Semences de Provence	a
Exeldur	1992	France	Valdur/Regal	Gae Maisse	d
Ixos	1990	France	Valnova/3/Tomclear/662//662	Verneuil Semences de Provence	d
Nefer	1996	France	164/Keops	Verneuil Semences de Provence	d
Neodur	1987	France	184-7/Valdur//Edmore	GAE-Maisse	d
Mexicali 75	1975	Mexico	61.130/Leeds//Jori"S"/3/GDOVZ469	CIMMYT-INIA	d
Altar 84	1984	Mexico	Ruff "S"/Flamingo "S"//Mexicali 75/3/SHWA"S"	CIMMYT-INIA	d
Aconchi 89	1989	Mexico	Altar 84/Araos	CIMMYT-INIA	e
Inrat 69	1969	Tunisia	Mahamoudi/Kyperounda	INRAT	f
Karim	1983	Tunisia	Jori"S"/Anhinga"S"//Flamingo"S"	CIMMYT-INRAT	f
Khiar	1992	Tunisia	Chen/Altar 84	CIMMYT-INRAT	f

a Ente Nazionale Sementi elette (ENSE), Milan, Italy
b Società Italiana Sementi (SIS), Bologna, Italy
c Istituto del Germoplasma (Germplams Institute), Bari, Italy
d Società Produttori Sementi (SPB), Bologna, Italy
e Istituto Sperimentale Cerealicoltura, Sezione di Foggia (Experimental Institute for Cereal Research), Foggia, Italy
f Institute Nationale de la Recherce Agricole, INRAT, Ariana,Tunisia
n.a.: not available

results from the EU Demonstration Project BIO4-CT97-2377 "Molecular marker systems for variety testing" were also considered (Röder et al. 2002; P. Donini and R. Cooke, unpublished results). PCR amplifications were carried out as described in Röder et al. (1998) and products were separated on 3% agarose gels (Seakem LE Agarose, BMA, Rockland, Me.). Sixty nine out of the 120 *Xgwm* loci (amplified by 65 WMS primer pairs) were selected based on their uniform distribution in the genome, profile quality and polymorphism level. The microsatellite *Taglut*, a trinucleotide repeat SSR (Devos et al. 1995), was also included because of its proven suitability for genotyping purposes (P. Donini, unpublished results).

PCR amplifications were carried out in a final volume of 25 μl as described in Röder et al. (1998), except for the forward primers which were fluorescently labelled; two different fluorochromes, IRD-700 and IRD-800 (MWG-Biotech, Ebesberg, Germany) were alternatively used. PCR reactions were electrophoresed in 25-cm-long denaturing 6% polyacrylamide (SequaGel XR, Polymed) gels in 1× TBE buffer. Real-time detection of alleles from multiplexed SSRs was achieved using the LI-COR dual-laser system. Amplicons were run on the LI-COR DNA Analysis 4200 Gene Read IR^2 automated genotyper (LI-COR, Lincoln, Neb.). For multiplexing PCR reaction products, 2 μl of each PCR product were pooled and diluted from 1:6 to 1:10 (regarding to the relative PCR product concentration) with IR^2 Stop Solution; 0.5 μl of the mix were loaded onto the gel. Alleles from different SSRs were discriminated on the basis of the differential labelling of the primers and molecular weights. The molecular weight of each allele was recorded by comparing the strongest of the corresponding stuttered bands with an IR-labelled molecular weight standard (IR Dye Sizing Standards, LI-COR, Lincoln, Neb.).

Data analysis

The gene diversity (discriminatory power) of each microsatellite locus was expressed as the diversity index (DI), which takes into account both the number of alleles present at a locus and the relative frequency of each allele:

$$DI = 1 - \Sigma p_j^2,$$

where p_j is the frequency of the j^{th} allele across all the 58 accessions (Weir 1990; Powell et al. 1996).

Following results and indications of previous studies (Matsuoka et al. 2002b), the proportion of loci with shared alleles was chosen as the most appropriate measure of genetic similarity. For each pair of accessions, the similarity value (GS_{ij}) was calculated according to Sneath and Sokal (1973) using the Simple Matching coefficient (SM) for multi-state qualitative data:

$$GS_{ij} = m/n,$$

where m = number of loci with allelic variants of the same molecular weight present in the two accessions *i* and *j* being compared, summed over all the surveyed loci, and n = total number of loci, excluding loci with missing data.

In some cases, microsatellite loci showed the presence of a residual non-uniformity within accessions (i.e. the presence of two different alleles per locus): in such cases the two alleles were considered as equally contributing to the genetic make-up of the accession. Taking into account this residual non-uniformity, the genetic diversity values were calculated as the exact proportion of loci with shared alleles between cvs by assigning two characters to each one of the 70 SSR loci. Principal coordinate analysis (PCoA) was performed from the genetic similarity matrix (Gower 1966). All computations were carried out with appropriate procedures of the software package NTSYS-pc ver. 2.0 (Rohlf 1997).

Results

SSR polymorphism

In total, 394 alleles were identified at the 70 polymorphic SSR loci. Polymorphism features of each SSR are reported in Table 2. These 70 SSR loci covered ca 80% of the A and B wheat genomes, as estimated from the microsatellite map obtained in bread wheat by Röder et al. (1998). The average number of allelic variants detected per locus ranged from two (*Xgwm164, Xgwm165-4A, Xgwm357* and *Xgwm415*) up to 12 (*Xgwm312*), with a mean of 5.63 ± 2.32. Two allelic variants were simultaneously detected in 3.6% of the investigated locus-genotype combinations (excluding missing data). A high frequency (i.e. >10%) of null alleles confirmed by two repeated PCR amplifications was detected at only five loci (*Xgwm126, Xgwm162, Xgwm282, Xgwm499* and *Xgwm611b*). DI values (the gene diversity estimates) of the 69 dinucleotide repeats ranged from 0.07 (*Xgwm67, Xgwm165-4A* and *Xgwm357*) to 0.80 (*Xgwm544* and *Xgwm611a*), with an average value of 0.56 ± 0.19. *Taglut*, the only trinucleotide repeat, revealed a DI value of 0.21 with four allelic variants, each one showing two distinct but co-migrating fragments, according to Devos et al. (1995).

Observed DI values have been reported as a function of the number of alleles/locus (Fig. 1) and were compared with the corresponding maximum theoretical DI values (mt-DI), i.e. the DI value corresponding to the highest possible diversity level: this comparison allowed us to assess the level of allelic imbalance present at each SSR locus. All the four bi-allelic loci had DI values considerably lower than their corresponding mt-DI (0.5), thus showing a strong allelic imbalance. Considering the relative DI value of each locus (the ratio of the DI value over the corresponding mt-DI), the 33 loci with three to five allelic variants showed lower values and a higher variation than the 32 loci with six to 12 allelic variants (64.6 ± 17.9% vs 75.1 ± 11.7%), thus indicating that the presence of allelic imbalance is more frequent at SSR loci with a low to medium number of alleles.

SSR loci showed differences as to the allele-size variation within a locus. Irrespective of the allelic richness of loci, in some cases (*Xwmg11, Xwmg304, Xwmg537, Xwmg544* and *Xwmg639-5B*) allele-size differences greater than two or three repeat units were rarely observed; these loci were characterized by low mean values of allele size differences. Other loci (*Xwmg122, Xwmg268, Xwmg291, Xwmg302, Xwmg408* and *Xwmg499*) showed a non-continuous, irregular distribution of allele-size differences, with the presence of high size differences among alleles.

Based on the sequencing information provided by Röder et al. (1998), among the 69 dinucleotide loci herein assessed, 40 of those can be considered as simple-perfect repeats, 17 as compound repeats and 12 as imperfect repeats, i.e. 29 irregular repeats in total (Table 2). Since imperfect and compound repeats are characterized by

Table 2 Characteristics of the 70 wheat microsatellite loci. Designation, chromosome location, centromere distance, sequence of the core repeat (as in Chinese Spring bread wheat: Devos et al. 1995; Röder et al. 1998), number of alleles across the 58 accessions, diversity index (DI) value, allele size range, size differences between alleles (mean and maximum values), and correlation coefficient of each SSR with the complete set of data. Loci highlighted in bold identify a core set of 20 primer combinations highly informative in cultivated durum wheat

SSR loci	Map position[a] Chrom.	Distance from centromere cM	Repeat class[b]	Motif	Allele number n	DI	Allele size range bp	Allele size differences		Correlation coefficient[c]
								Mean	Max.	
Xgwm136	1AS	39	p	CT	11	0.76	278–370	9.2	26	0.34 ***
TAGLUT	1AS	–	c	CAG/CAA	4	0.23	144–160	5.3	8	0.29 ***
Xgwm164	1AS	2	p	CT	2	0.21	125–129	2.0	2	0.05 ns
Xgwm 357	1AL	9	p	GA	2	0.07	118–120	2.0	2	0.04 ns
Xgwm 99	1AL	96	p	CA	7	0.46	112–142	5.3	14	0.20 **
Xgwm 18	1BS	6	c	CA/TA	3	0.40	186–190	2.0	2	0.20 **
Xgwm 11	1BL	2	c	TA/CA	5	0.57	214–226	2.5	4	0.35 ***
Xgwm 268	1BL	74	i	GA	5	0.57	204–232	7.0	16	0.48 ***
Xgwm 636	2AS	95	i	GA	8	0.76	87–113	3.7	10	0.36 ***
Xgwm 95	2AS	5	p	AC	7	0.65	110–131	3.3	10	0.40 ***
Xgwm 448	2AS	5	p	GA	9	0.75	220–252	4.0	8	0.48 ***
Xgwm 122	2AL	3	c	CT/CA	6	0.59	128–205	15.4	52	0.43 ***
Xgwm 294	2AL	14	i	GA	4	0.47	72–110	12.7	30	0.43 ***
Xgwm 312	2AL	14	p	GA	5	0.47	182–235	8.0	10	0.40 ***
Xgwm 148	2BS	19	p	CA	6	0.63	146–168	4.8	18	0.19 **
Xgwm 120	2BL	19	c	CT/CA	5	0.58	128–160	10.0	18	0.29 ***
Xgwm 526	2BL	76	p	CT	6	0.57	140–156	3.6	6	0.33 ***
Xgwm 619	2BL	106	p	CT	5	0.59	142–168	6.5	10	0.48 ***
Xgwm 5	3AL	4	c	TC/GT/GA	7	0.77	156–174	3.0	4	0.36 ***
Xgwm 155	3AL	64	p	CT	3	0.35	127–130	1.5	2	0.36 ***
Xgwm 162	3AL	91	i	CA	5	0.54	200–204	2.0	2	0.23 ***
Xgwm 493	3BS	127	i	CA	3	0.50	138–180	21.0	30	0.34 ***
Xgwm 389	3BS	118	c	CT/GT	6	0.47	116–132	3.2	6	0.14 *
Xgwm 566	3BL	4	c	CA/GA/TA	3	0.47	126–134	4.0	6	0.18 **
Xgwm 247	3BL	132	p	GA	10	0.76	137–195	5.8	20	0.23 ***
Xgwm 165-4A	4AS	8	p	GA	2	0.07	188–193	5.0	5	0.07 ns
Xgwm 610	4AL	17	i	GA	6	0.48	154–170	3.2	6	0.38 ***
Xgwm 637	4AL	40	p	CA	6	0.75	157–173	2.8	9	0.28 ***
Xgwm 160	4AL	85	p	GA	3	0.57	188–198	5.0	8	0.28 ***
Xgwm 368	4BS	7	p	TA	6	0.75	252–282	5.2	10	0.42 ***
Xgwm 513	4BL	8	p	CA	4	0.60	150–156	2.0	2	0.27 ***
Xgwm 165-4B	4BL	16	p	GA	6	0.73	252–266	2.8	4	0.39 ***
Xgwm 251	4BL	30	p	CA	4	0.57	96–118	7.3	16	0.10 ns
Xgwm 6	4BL	50	p	GA	10	0.70	174–248	8.0	20	0.27 ***
Xgwm 304	5AS	29	p	CT	6	0.67	198–210	2.4	4	0.26 ***
Xgwm 415	5AS	29	i	GA	2	0.10	136–138	2.0	2	0.04 ns
Xgwm 154	5AS	15	i	GA	4	0.54	118–134	4.7	10	0.39 ***
Xgwm 156	5AL	19	p	GT	6	0.69	288–320	6.8	24	0.11 ns
Xgwm 639-5A	5AL	60	p	GA	3	0.20	135–143	4.0	6	0.00 ns
Xgwm 126	5AL	133	p	CA	4	0.50	193–207	5.3	8	0.03 ns
Xgwm 291	5AL	150	p	CA	5	0.70	140–166	6.5	18	0.22 **
Xgwm 234	5BS	37	c	CT/CA	6	0.70	228–262	7.2	18	0.33 ***
Xgwm 544	5BS	12	c	CT/GT	10	0.79	174–204	2.7	6	0.33 ***
Xgwm 213	5BL	0	p	GA	11	0.63	151–233	8.4	26	0.33 ***
Xgwm 67	5BL	0	p	CA	3	0.07	93–97	2.0	2	0.26 ***
Xgwm 499	5BL	29	p	GA	7	0.59	124–184	8.3	32	0.28 ***
Xgwm 639-5B	5BL	36	p	GA	5	0.43	172–182	2.5	4	0.31 ***
Xgwm 408	5BL	74	c	CA/TA	4	0.52	149–183	11.3	28	0.22 ***
Xgwm 459	6AS	67	p	GA	6	0.64	115–182	11.4	26	0.24 ***
Xgwm 570	6AL	31	c	CT/GT	5	0.57	104–147	10.8	37	0.12 ns
Xgwm 169	6AL	50	p	GA	5	0.54	185–203	4.5	8	0.11 ns
Xgwm 518	6BS	34	p	CA	5	0.66	150–162	3.0	4	0.38 ***
Xgwm 193	6BS	7	c	CT/CA	4	0.64	167–179	4.0	8	0.50 ***
Xgwm 88	6BS	3	c	GT/GA	8	0.77	130–152	3.1	6	0.51 ***
Xgwm 680	6BS	0	c	GT/GA	4	0.65	128–140	4.0	8	0.48 ***
Xgwm 219	6BL	40	i	GA	6	0.69	154–188	6.0	10	0.26 ***
Xgwm 60	7AS	52	p	CA	5	0.62	188–220	8.0	18	0.27 ***
Xgwm 573-7A	7AS	21	p	CA	4	0.50	179–191	4.0	6	0.17 *
Xgwm 276	7AL	28	p	CT	8	0.62	101–135	4.9	10	0.17 *
Xgwm 282	7AL	48	p	GA	7	0.47	170–224	8.7	16	0.38 ***
Xgwm 332 a	7AL	48	p	GA	5	0.44	206–240	7.7	15	0.35 ***
Xgwm 332 b	7AL	48	p	GA	3	0.13	188–198	5.0	8	0.29 ***

Table 2 (continued)

SSR loci	Map position[a] Chrom.	Distance from centromere cM	Repeat class[b]	Motif	Allele number n	DI	Allele size range bp	Allele size differences		Correlation coefficient[c]
								Mean	Max.	
Xgwm 537	7BS	43	c	CA/TA	7	0.71	203–223	2.7	4	0.26 ***
Xgwm 573-7B	7BS	33	p	CA	4	0.68	218–224	2.0	2	0.32 ***
Xgwm 46	7BS	18	i	GA	8	0.58	171–189	2.6	4	0.39 ***
Xgwm 297	7BS	5	c	GT/GA	5	0.49	164–174	3.0	4	0.27 ***
Xgwm 302	7BL	23	p	GA	12	0.77	220–380	13.3	40	0.41 ***
Xgwm 577	7BL	105	c	CA/TA	8	0.74	136–222	12.3	38	0.44 ***
Xgwm 611 a	7BL	114	i	GA	10	0.80	150–180	3.3	8	0.33 ***
Xgwm 611 b	7BL	114	i	GA	5	0.64	142–158	4.0	8	0.44 ***

[a] Map position of SSR loci is as reported in Röder et al. 1998, except for *Xgwm 619* (Korzun et al. 1999) and *Xgwm 332-b* and *Xgwm 611-b* (Peng et al. 2000)
[b] p: perfect repeats, c: compound repeats, i: imperfect repeats, as indicated by Röder et al. (1998) for Chinese Spring bread wheat
[c] Correlation coefficients computed between the similarity matrix based on each microsatellite locus and the similarity matrix based on all the 70 loci analysed
*, **, ***: significant at P 0.05, P 0.01 and P 0.001, respectively; ns: not significant

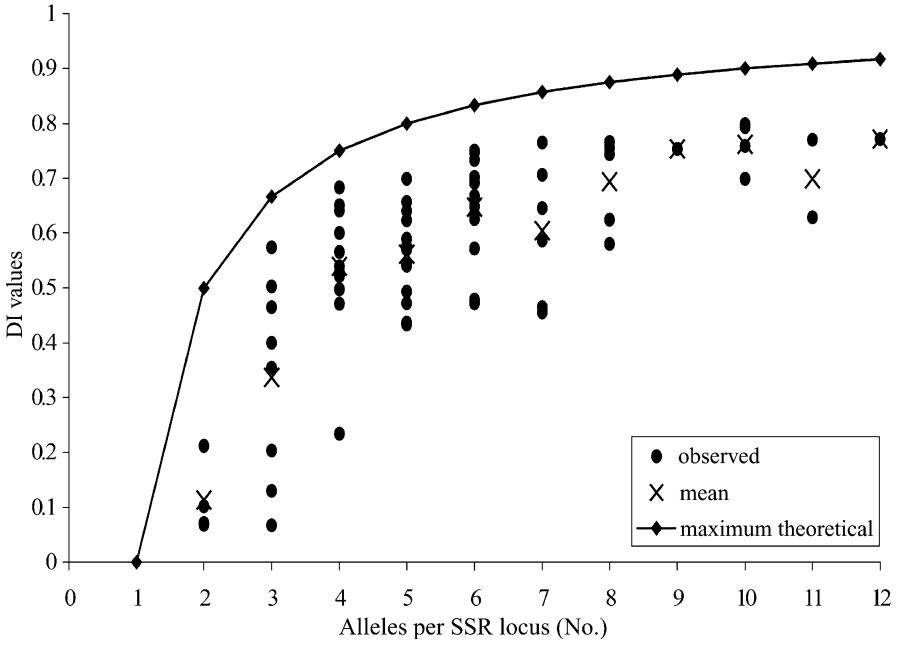

Fig. 1 Scatterplot of diversity index (DI) values as a function of allele number for the 70 SSR loci analysed across the 58 durum wheat accessions. Mean and maximum theoretical DI values have also been plotted

similar mutational and evolutionary features (Murray 1996), data from these two repeat classes were pooled and compared with polymorphism features of perfect repeats. No significant differences were found between simple-perfect and irregular repeats (t test) as to the mean number of alleles per locus (5.7 and 5.6, respectively) and mean DI values (0.54 and 0.59, respectively). However, the two main classes differed as to their variances in diversity values: variances in allele number per locus and DI values were lower within irregular repeats than within simple-perfect repeats (variances equal to 4.11 and 6.47, P = 0.11, F test, for the number of alleles per locus and 0.022 and 0.042, P = 0.035, for DI values, respectively). In particular, most of the SSR loci with a low polymorphism content (i.e. with two to three alleles) were found among the simple-perfect repeat loci. Among irregular repeats, compound repeats (17 loci) showed the lowest variances for polymorphism indices (i.e. 3.62 for the number of alleles per locus and 0.014 for DI values).

Simple-perfect, imperfect and compound repeats did not differ significantly (F test) as to the allele-size variation within the locus (mean values equal to 5.3, 6.0 and 6.0 bp, respectively). Simple-perfect repeats included 25 GA, 14 CA and 1 TA repeats. GA and CA groups showed a fairly similar mean number of alleles per locus (6.1 and 5.0, respectively; P = 0.06, t test) and DI values (0.51 and 0.58, respectively; P = 0.14); however, GA repeats, as compared to CA repeats, showed a notably higher variance for number of alleles (9.24 as compared to 1.54, respectively) and for DI values (0.049 and 0.028, respectively).

As compared to the 33 dinucleotide SSRs of the A genome, the 36 SSRs mapping in the B genome had a higher mean number of alleles (5.2 and 6.1, respectively; $P = 0.05$, t test) and a significantly higher mean DI value (0.50 and 0.61, respectively; $P = 0.01$, t test).

In order to assess whether the chromosome position affected the polymorphism level, the 69 dinucleotide repeats were assigned to two groups according to their distance from the centromere (see Dvorák et al. 1998) and were compared for polymorphisms. The 18 proximal loci (with distance from centromere $\leq$ 10 cM) had a slightly lower mean polymorphism content, yet not significantly different than that of the 51 distal loci (mean number of alleles: 5.1 vs 5.9, $P = 0.10$, and mean DI value: 0.51 vs 0.58, $P = 0.13$, t test, respectively). Correlation values between the distance of loci from the centromere and the polymorphism level were low (r values equal to 0.07 and 0.13 as to the number of alleles and DI values, respectively), and correlations did not improve even considering the relative distance from the centromere.

Assessment of genetic similarities by SSR loci

Genetic similarity (GS) estimates of all possible (1,653) pairs of accessions ranged from 0.15 (Russello SG7 vs Aconchi 89) to 1.0 (Vitron vs Vitromax), with a mean value of 0.44. GS estimates for the B genome data (mean GS = 0.38) were notably lower than those obtained with the A genome data (mean GS = 0.49).

Coefficients of correlation between the similarity matrices generated by each locus and the reference matrix obtained from all marker data ranged from 0.00 to 0.51, with a mean of 0.30 (Table 2). Some loci (e.g. *Xgwm193, Xgwm268* and *Xgwm619*) with a mean number of alleles ranging from four to five were characterized by correlation values with the reference matrix (r = 0.48–0.50) higher than those (r = 0.23–0.34) of other loci (e.g. *Xgwm136, Xgwm213* and *Xgwm247*) that ranked at the top for allelic richness (up to 11 alleles/SSR). A core set of 20 highly informative WMS primer combinations (amplifying 23 high quality *Xgwm* loci evenly distributed on the wheat map) has been selected among those tested (Table 2). The genetic diversity values obtained with this core-set of markers showed a correlation value with the genetic diversities obtained from all loci equal to 0.91.

Rare- and founder-allele distribution: contribution of founders to the genetic diversity of modern varieties

GS values among a group of key-genotypes (the old Mediterranean accessions Russello SG7, Saragolla and Inrat 69, and the group of ten founders) are reported in Table 3. Russello SG7, Saragolla and Inrat 69 showed the lowest levels of similarity when compared with all the ten important founders of the modern gene pool. Russello SG7 had a genetic profile clearly different from those of the ten founders (GS values ranging from 0.16 to 0.28),

Table 3 Contribution of founder genotypes to the elite durum germplasm herein analysed (45 cvs) and SSR-based genetic similarity (GSij) values among three accessions (columns 9–17). Mean GS values (column 18) of each founder genotypes vs all the 45 elite cultivars have been reported

Founder	Year of release	Novel alleles[a] No.	Genetic variation accounted for by foundation genotypes		Russello SG7	Saragolla	Inrat 69	Cappelli	Capeiti 8	Appulo	Valnova	Valforte	Creso	Mexicali 75	Karim	Latino	Mean GS
			%[b]	%[c]													
Cappelli	1915	72	41.5	44.1	0.28	0.55	0.49										0.42
Capeiti 8	1940	33	11.2	25.4	0.25	0.41	0.43	0.54									0.38
Appulo	1964	9	1.1	10.9	0.24	0.38	0.41	0.56	0.75								0.35
Valnova	1975	29	15.2	42.5	0.22	0.37	0.30	0.55	0.40	0.45							0.51
Valforte	1980				0.22	0.37	0.33	0.56	0.43	0.47	0.92						
Creso	1974	23	7.7	22.9	0.25	0.33	0.33	0.46	0.41	0.36	0.38	0.39					0.45
Mexicali 75	1975	14	5.0	24.9	0.16	0.31	0.25	0.40	0.32	0.38	0.62	0.65	0.41				0.50
Karim	1983	21	7.6	26.7	0.20	0.22	0.29	0.35	0.30	0.21	0.38	0.41	0.51	0.49			0.49
Latino	1982				0.24	0.25	0.30	0.33	0.24	0.23	0.31	0.34	0.46	0.39	0.85		
Altar 84	1984	14	2.5	12.2	0.16	0.27	0.23	0.31	0.24	0.21	0.41	0.41	0.41	0.56	0.44	0.44	0.46
Total		215	91.8	209.6													

[a] Number of novel alleles first introduced in the germplasm by each founder; alleles contributed by Valnova and Valforte and by Karim and Latino have been pooled, respectively, due to the high genetic similarities of these genotypes

[b] Calculated over all the 70 SSR loci investigated

[c] Calculated over the specific loci carrying the novel alleles

while Saragolla and Inrat 69 showed moderate similarity only with the oldest Mediterranean founder Cappelli (GS values of 0.55 and 0.49, respectively). Among founders, Cappelli showed moderate similarities as to all the other founders subsequently introduced (GS ranging from 0.31 to 0.56). In contrast, Capeiti 8 and Appulo founders showed similarities with the recently released modern founders (i.e. Mexicali 75, Karim-Latino and Altar 84, with values ranging from 0.21 to 0.38) lower than those observed for Cappelli (from 0.31 to 0.40).

The recent founders (Valnova-Valforte, Creso, Mexicali 75, Karim-Latino and Altar 84) were moderately related (mean GS = 0.45). As expected from pedigree information, Valnova and Valforte, selected within the same breeding program, were very similar to each other (GS = 0.92). In keeping with the pedigree information, a clear genetic relationship was also detected between Valnova-Valforte and Mexicali 75 (mean GS = 0.63). The first important founders characterised by the modern plant type, i.e. Valnova-Valforte and Creso, were only moderately related (mean GS = 0.36), while medium levels of similarity were observed among the more recently released CIMMYT founders (Mexicali 75, Karim and Altar 84), with similarities ranging from 0.44 (Karim-Altar 84) to 0.49 (Karim-Mexicali 75).

Out of the 394 different alleles detected in the 58 accessions, 168 alleles (43% of the total) were classified as rare alleles, due to their low frequency across accessions (i.e. <5%, Russell et al. 2000). Rare alleles were found across all chromosomes (data not reported). Russello SG7, Saragolla and Inrat 69 showed a richness in rare alleles (35, 20 and 19, respectively), differently from Cappelli and Capeiti 8 (with only five and four rare alleles, respectively) that, while belonging to the same Mediterranean germplasm pool of Saragolla and Inrat 69, are instead recognized as important "founder-genotypes" of the modern gene pool herein analysed.

After excluding the three landrace-derived accessions, which did not contribute notably to the foundation of the modern elite germplasm, a total of 342 different alleles were detected, 112 of which (32.4%) were classified as rare. The ten founders contributed a total of 215 alleles (62.8%), with only 21 considered as rare alleles, and 92.7% of the total molecular variation detected in the 45 modern cvs traced back to these alleles. A large portion (41.5%) of the molecular variation detected among the 45 modern cvs can be traced back to alleles present in the oldest founder (true ancestor) Cappelli (Table 3). In the modern germplasm, these Cappelli-like alleles were detected throughout the A and B genomes at most of the loci analysed. The 33 alleles differentiating Capeiti 8 (Cappelli/Eiti) from Cappelli accounted for 11.2% of the total genetic variation among modern varieties.

When considering specific alleles possibly introduced in the modern germplasm for the first time by each of the relevant founders, the highest contribution to the total genetic variation present in the modern germplasm can be attributed to Cappelli (see Table 3), which is also the oldest among the founders considered in this study. This notwithstanding, important more recent founders such as Valnova-Valforte and Karim-Latino showed mean GS values with modern cvs (GS = 0.51 and 0.49, respectively) higher than that detected for Cappelli (GS = 0.42). The level of diffusion in the modern germplasm of the novel alleles contributed by these outstanding varieties was comparable to that of the Cappelli-like alleles (Table 3).

Principal coordinate analyses

Genetic relationships among the 58 accessions were investigated by means of principal coordinate analysis (PcoA; Fig. 2). The first (PCo1) and second (PCo2) principal coordinates accounted for 16.0 and 9.2% of the total variation, respectively, while the third coordinate (data not shown) accounted for a further 6.9% of the variation, bringing the total explained variation up to 32.3%.

Based on the known pedigree information, five main groups of genotypes can be identified in the diagram, highlighted by encircling the most diverse genotypes within each of the five main groups. Group 1 includes genotypes all related to the old germplasm from the Mediterranean basin, mainly northern Africa and southern Italy. This group was positioned in the lower right corner of quadrant IV showing a restricted and scarcely overlapping nature of diversity as regards to the majority of the modern cvs. Mean genetic similarity values pointed out that Saragolla, Inrat 69 and particularly Russello SG7 were the most diverse accessions (mean GS of Russello SG7 with the other 57 cvs = 0.22).

PCo1 revealed a clear differentiation of the more recent CIMMYT-derived cvs (subgroups 2C and 2D), clustered on the left side of the diagram, from the gene pools positioned on the right side of the diagram, i.e. the old Mediterranean accessions (group 1) and the subgroups of cultivars related to the former CIMMYT-derived founders, namely Valnova-Valforte and Creso (subgroup 2A and 2B, respectively).

PCo2 effectively separated cvs of group 1 (quadrant IV) from the two closely related founders Valnova-Valforte, positioned in quadrant III together with some derived cvs (Mexicali 75 and Grazia). An important group of modern successful varieties (Fortore, Gargano, Ofanto, Platani and Simeto) well-adapted to Southern Italy are positioned between the parents Valnova-Valforte and Capeiti 8-Appulo, respectively, while being classified closer to the former ones. PCo2 effectively separated Creso from the Valnova-Valforte founders and the related groups.

The results of PCoA indicate that, among groups of modern cvs, the most notable variation pertains to the group of varieties somehow related to the "Val"-founders. Cvs of subgroups 2C and 2D, identified by the corresponding outstanding CIMMYT-related materials Karim-Latino (JO/AA//FG derived cvs) and Altar 84, overlapped in a restricted diversity area in the middle-left portion of the diagram. The two breeding lineages are clearly

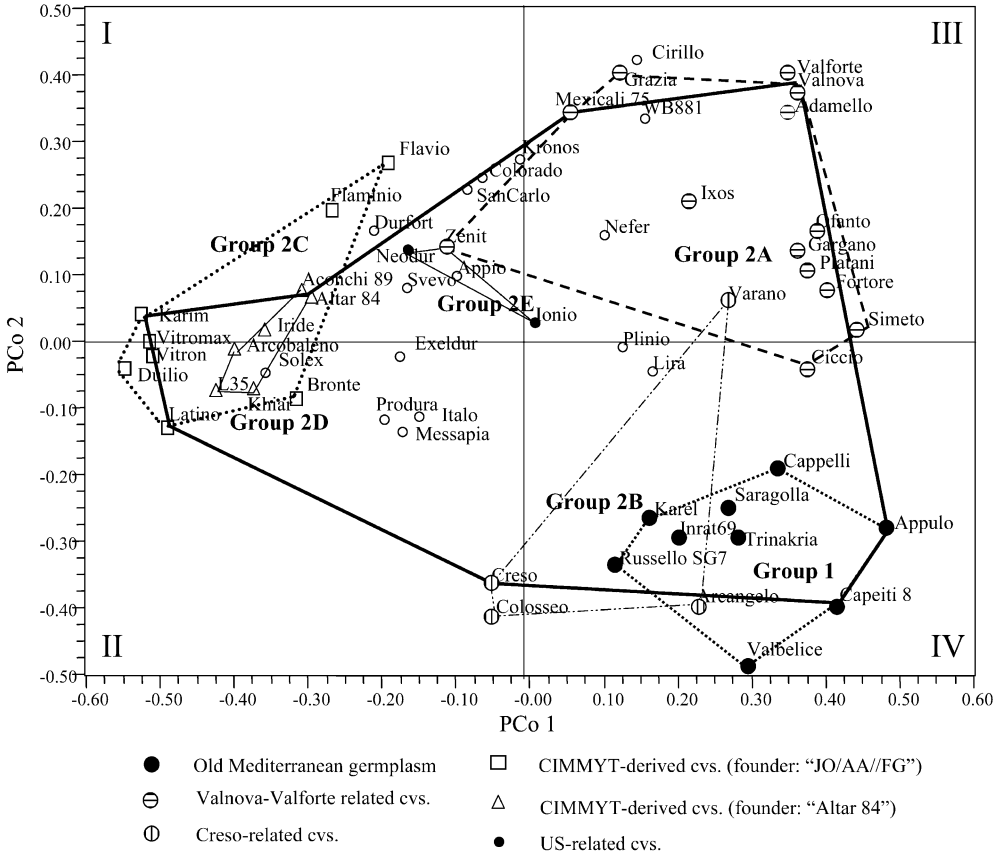

Fig. 2 Associations among the 58 durum wheat accessions as revealed by principal coordinate analysis. Principal coordinate 1 and 2 have been depicted. Convex hulls were drawn around the most diverse cultivars related to each of five main breeding groups or gene pools. **Group 1**: the old Mediterranean germplasm; **group 2A**: Valnova-Valforte related cvs; **group 2B**: Creso-related cvs; **group 2C**: "JO/AA//FG"-related cvs; **group 2D**: Altar 84-related cvs; **group 2E**: US-related cvs. The **thick line** encircles the most diverse of the ten foundation-genotypes

distinguished only by PCo3. The small subgroup 2E includes Italian (Ionio and Zenit) and French (Neodur) cvs having a common origin, tracing back to the North Dakota germplasm (cvs Edmore and Vic). The main founders were generally positioned near to the borders of the diagram, thus accounting for a large portion of the total variation, while a number of modern varieties of mixed origin were spread across the centre of the diagram.

Discussion

Variability of SSR loci

The 69 selected dinucleotide *Xgwm* loci with an additional trinucleotide locus (*Taglut*) provide an effective "genotyping set" (Macaulay et al. 2001) due to their high quality, informativeness and genome coverage. The mean number of allelic variants per locus (5.63), the mean DI value (0.56) and the average GS_{ij} among accessions (0.44) indicate a level of diversity similar to that reported by SSR-based studies in other small grain cereals, in particular bread wheat. In fact, in the European elite bread wheat germplasm assessed for variation at *Xgwm* loci, Plaschke et al. (1995) and Stachel et al. (2000) found from 5.2 to 6.2 alleles and genetic similarities of approximately 0.40, analysing different gene pools. Manifesto et al. (2001) profiled Argentine elite bread wheat cvs with ten SSRs selected for high polymorphism and observed a variation in allele number and DI per locus similar to that revealed in the durum accessions of our study, while in a large pool of elite European bread wheat cvs (500 cvs), recently analysed with SSRs, the average number of alleles per locus resulted in 10.5 (Röder et al. 2002).

The potential of SSRs for detecting polymorphism in *Triticum* was clearly shown by Huang et al. (2002), who detected 18.1 allelic variants per SSR locus among bread wheat accessions from the Gatersleben germplasm collection.

In durum wheat, a genetic diversity study in cultivated materials was reported by Sorrells et al. (1995) and by Autrique et al. (1996), who used RFLPs (restriction fragment length polymorphisms) to analyse a collection of 113 accessions including landraces and advanced materials (mainly from the CIMMYT/ICARDA breeding program). In these studies, RFLPs detected an average of 4.3 variants per probe and classified most of the improved materials in a unique subcluster separated from the majority of the landrace accessions; improved materials were also characterised by a relatively low diversity level (mean Nei's genetic diversity equal to 0.21). Only recently, SSRs have been exploited to investigate genetic relationships in cultivated durum wheat: Dograr et al. (2000), using seven dinucleotide SSRs, identified from

five to 13 different alleles per locus and an overall mean DI of 0.76 across Turkish cvs, while Eujail et al. (2002) reported a mean of 5.5 alleles per locus with 11 *Xgwm* loci used to screen 64 durum accessions with a broad genetic basis (including landraces, varieties and breeding lines, mainly chosen among those previously analysed by Autrique et al. 1996).

As compared to results reported with durum landraces and/or populations of wild emmer wheat *Triticum dicoccoides* Korn, our as well as other studies evidenced a lower genetic diversity in cultivated tetraploid wheats. In fact, in a sample of Ethiopian tetraploid wheats (26 *Triticum durum* and *T. turgidum* landraces), 12 *Xgwm* loci revealed 7.9 alleles per locus (Messele 2001), while Fahima et al. (2002) reported a mean of 18 allelic variants per locus in wild emmer wheat populations profiled with 20 *Xgwm* loci.

The correspondence between the level of diversity observed in our survey and in the study of Eujail et al. (2002), as revealed by the analysis of the results from the seven *Xgwm* loci common to both studies (the correlation values calculated for the number of alleles and for the DI values were equal to 0.64 and 0.57, disregarding the contrasting results observed for the *Xgwm169* locus) is most likely due to the relatedness of the modern durum wheat materials assayed in both studies. A moderate correlation ($r = 0.56$) was also found over 11 common *Xgwm* loci between the number of alleles detected in our study, mainly including modern varieties, and that reported by Messele et al. (2001) in Ethiopian durum landraces. These results indicate that the polymorphism level of microsatellite loci is rather stable across different durum wheat gene pools. Loci with a relatively high polymorphism (more than 5–6 allelic variants) in this study should thus be considered as valuable and informative marker loci, suitable for further molecular studies targeting the durum wheat germplasm.

Dinucleotide repeats with a low number of allelic variants and/or a low relative DI value could possibly mark chromosome regions where selection for agronomically valuable alleles reduced the genetic diversity. Chromosome regions characterised by a low variation at neutral loci were also observed in similar SSR-based surveys in barley (Russell et al. 2000) and wheat (Stachel et al. 2000; Huang et al. 2002). In our study, the relatively high frequency of microsatellites with a lower than expected genetic diversity due to their high allelic imbalance could be attributed to the presumably high linkage disequilibrium level typical of the elite modern gene pool, particularly in self-pollinating species like durum wheat (Nordborg et al. 2002). Moreover, recent database analyses on the abundance and relative distribution of microsatellite loci, carried out on different plant species including rice, maize and wheat (Morgante et al. 2002), pointed out that even dinucleotide repeats show a significant association with the non-repetitive, gene-rich DNA fraction of plant genomes and are especially frequent in the regulatory untranslated portions of the transcribed regions of the genome (5′ and 3′ UTRs). Most of the *Xgwm* loci have been obtained from the hypomethylated, gene-rich portion of the wheat genome (Röder et al. 1998); Morgante et al. (2002), in their extensive database evaluation, reported that GA/CA repeats, similar to those used in this survey, were found at higher frequencies in EST regions as compared to genomic regions.It should be possible to obtain further insights on the human-driven evolution of the durum wheat germplasm by profiling with such SSRs a wider range of historical ancestor-genotypes, in a retrospective genetic diversity analysis. The molecular characterisation of the main durum wheat cultivars, landraces, ancestor genotypes and also accessions which did not significantly contribute to the foundation of the modern germplasm with the less variable SSRs found in this study should allow for the comparison of the gene-diversity values and allelic frequencies among different gene pools that presumably underwent different selection pressures. This approach is currently being explored in maize, barley and wheat in projects aiming at the molecular and phenotypic characterization of wide germplasm collections (Russell et al. 2000; Vigouroux et al. 2002). Some interesting examples of microsatellite loci with marked differences in polymorphism content when assessed in different durum wheat gene pools were identified comparing our results with those of Eujail et al. (2002; e.g. the *Xgwm 169* locus) and Messele (2001, e.g. *Xgwm357*).

Features of dinucleotide repeats

As microsatellite loci are characterised by peculiar mutation mechanisms (Goldstein and Schlötterer 1999; Chambers and MacAvoy 2000), a better knowledge about their mutation pattern and rate would be valuable for data analysis and interpretation. A concern about using SSRs in diversity analyses relates to the possible occurrence of allelic homoplasy (i.e. alleles with similar size but originated by different mutation events), a phenomenon leading to misinterpretations and/or overestimations of relatedness. The frequency of homoplasy is expected to be higher for microsatellite markers than for other marker classes (Murray 1996). The highly polymorphic dinucleotide repeats have shown a complex mutation process that varies greatly among loci (Macaubas et al. 1997; Colson and Goldstein 1999; Barrier et al. 2000) and that cannot exclusively be ascribed to polymerase slippage events, considered as the major factors responsible for allelic homoplasy (Murray 1996; Estoup and Cornuet 1999). In order to limit the possible bias due to homoplasy, we have focused on dinucleotide repeat loci rather than trinucleotide loci: in fact, the molecular variation revealed by trinucleotide SSRs, as compared to dinucleotides, is usually lower (Cho et al. 2000; Scott et al. 2000) and more strictly adherent to the step-wise mutation model (Murray 1996). Trinucleotides were thus considered to be less suited to perform a genetic diversity study in a set of related accessions such are those herein considered.

Recent genetic diversity and phylogenetic analyses in maize based on SSRs revealed their complex mutation patterns using genetic distances based on the proportion of shared alleles rather than measures of genetic distance related to the stepwise assumption (Matsuoka et al. 2002a, b). Allele size information (such as the variation of allele size differences among alleles within a locus) highlights the level of complexity of the mutational processes at the microsatellite locus. A number of loci tested in our survey were characterised by a high-allele size variation and our results agree with those of Huang et al. (2002) in bread wheat. These SSRs possibly identify loci where mutational mechanisms other than replication slippage in the core repeat may have occurred, or loci with a high mutation rate. Thus, dinucleotide loci with a relatively high allele size variation should be preferentially considered in order to minimize the occurrence of homoplasy events (Murray 1996); however, the use of dinucleotides with high-allele size variations requires high accuracy in the molecular analysis.

Polymorphism level as related to chromosome location

An overall reduction in polymorphism is expected in the low recombining, pericentromeric regions, especially in self-fertilizing species and even more so in elite modern materials which have experienced strong foundation effects and have undergone repeated cycles of selection. Dvorák et al. (1998) profiled six *Aegilops* species with RFLPs in order to study the relation between map position, recombination rates and gene diversity: they observed a significant correlation between the gene diversity and the coefficient of exchange (an estimate of the local recombination rate), particularly in pericentromeric loci within 10 cM from the centromeres, which showed low recombination levels and diversity values. Our results, using microsatellite loci, do not support this finding and are more in line with the report of Ramsay et al. (2000) with SSRs in barley. These authors suggested that the relatively high mutation rate of SSRs might have counterbalanced the reduction of neutral variation due to selection effects occurring on the pericentromeric regions with lower recombination levels. No obvious reduction of diversity in these regions was also observed by Tenaillon et al. (2001) in maize. However, as underlined by Dvorák et al. (1998) and by Tenaillon et al. (2001), the recombination rate along chromosomes is highly variable and difficult to be precisely determined in cereals, particularly in wheat (Gill et al. 1996a, b).

Polymorphism level of the different dinucleotide repeat classes

In this study, the set of dinucleotide repeats sampled covered all the three main microsatellite classes, namely simple-perfect, compound and imperfect repeats, with relative proportions of each class similar to those previously reported by Röder et al. (1998) for a large set of these dinucleotide repeats. According to such findings, simple-perfect repeats were more frequently assayed in the A genome, compound repeats were preferentially represented in the B genome, while imperfect repeats were more uniformly distributed among genomes. Among simple-perfect repeats, GA motifs were preferentially chosen over CA motifs, in keeping with the observations reported in wheat and maize (Ma et al. 1996; Röder et al.1998; Morgante et al. 2002). Although our results did not show significant differences in the average polymorphism among the three classes, compound and imperfect repeats appear as more valuable markers, because of their lower variance in allele number and DI value (especially compound repeats). In fact, the preferential use of compound and imperfect repeats should reduce the risks of sampling loci characterised by a low average number of repeats (short microsatellites) which in general correlates with low diversity levels. However, it should be noted that the compound repeats used herein were mostly from the B genome (Röder et al. 1998).

Contrasting results have been reported on the variability of complex, irregular repeats, which are expected to show a higher portion of invariant markers and a lower mean mutation rate and polymorphism as a consequence of the stabilizing effect of the array interruptions (Taylor et al. 1999). However, Colson and Goldstein (1999) reported different results. Notably, among the 12 imperfect loci assayed herein, a rather low level of diversity was only detected for *Xgwm415*, while among the 17 compound dinucleotides only two loci showed a number of alleles lower than four. Guyomarc'h et al. (2002), characterising bread wheat cvs with a recently developed set of D-genome specific dinucleotide SSRs, did not find significant differences between compound and simple microsatellites as to their mean polymorphism level, and even the presence of interruptions within the core-repeats did not negatively affect the polymorphism level. Among perfect repeats, the CA group appeared to be characterised by an overall more uniform level of polymorphism than the GA group; in this case, no interference effects should be attributed to the genome, since the two groups were homogeneously distributed across the A and B genomes. Among perfect repeats, CA motifs thus seem to be the best choice for diversity analyses, at least in the set of germplasm herein analysed.

Polymorphism level of the A and B genomes

The higher values of genetic diversity obtained with the SSRs mapping to the B genome reflect the higher polymorphism level of these loci as compared to those on the A genome. A higher polymorphism level of the B genome was already detected in wheat, as indicated by the higher than average number of RFLP, AFLP and SSR markers mapping in the B genome (Marino et al. 1996; Röder et al. 1998; Lotti et al. 2000; Nachit et al. 2001). Peng et al. (2000) in a high-resolution *T. durum* × *T.*

dicoccoides map found that the B genome chromosomes had a significantly higher density of AFLP polymorphisms as compared to the A genome. A number of cytogenetic and molecular investigations pointed out that the B genome is the most differentiated and divergent from the ancestral donors among the three wheat genomes (Zohary and Feldman 1962; Blake et al. 1999; Huang et al. 2002; Zhang et al. 2002). In a survey for RFLP polymorphisms across six *Aegilops* species, Dvorák et al. (1998) found the lowest allele frequency and the highest gene diversity in the cross-fertilizing *Aegilops speltoides* (the most-likely donor of the wheat B genome, among the *Sitopsis* species). This could account for the higher diversity content of the B genome as compared to the A genome, especially in terms of mean DI value, rather than of the mean number of alleles. Also, the B genome chromosomes appear to be relatively rich, as compared to the A genome ones, in various classes of repetitive DNA, particularly microsatellites (Gerlach and Peacock 1980; Cuadrado and Schwarzacher 1998). Stachel et al. (2000) and Huang et al. (2002), in similar surveys of *Xgwm* dinucleotide loci on bread wheat, did not show marked differences in the overall genetic diversity of the A and B genomes; however, as compared to our study, a lower number of loci (28 and 24 respectively) was assayed in the former studies.

Patterns of molecular variation among the elite durum wheat germplasm

In general, the hierarchical subdivision of the germplasm herein analysed with SSRs agreed with the pedigree information. The comparison of the profiles of Russello SG7, Saragolla and Inrat 69, the three old accessions representative of the native Mediterranean germplasm, with those of the 45 modern cvs does not support an appreciable inheritance of the allele signatures found in the ancient germplasm locally cultivated before the introduction of modern breeding practices. However, such germplasm should be considered as a valuable source for recovering potentially valuable genetic diversity, in particular for grain quality and tolerance to biotic and abiotic stresses.

The reduction of the genetic diversity which occurred at the foundation of the modern gene pool is evident from the observation that as few as two important ancestors (namely Cappelli and Eiti), directly derived from landraces of different origin (Bozzini et al. 1998), accounted for a relevant portion of the total diversity present in the subsequent founders and in the modern germplasm. Within the main breeding groups identified by the CIMMYT-derived founders, molecular similarities higher than those expected from co-ancestries were observed between each of the main recent founders and the corresponding modern derived cvs, as in the cases of the founders Valnova-Valforte, Creso, Latino-Karim and Altar 84. These results could possibly be ascribed to high selection pressures coupled with the adoption of breeding schemes (e.g. the pedigree method) that do not allow for extensive recombination, as suggested by a similar study in barley (Graner et al. 1994). Selection will reduce the correlation between molecular and pedigree-based estimates (Lynch 1988). Particular marker alleles, contributed by each of the main founders, tagged chromosome regions preferentially selected over time, thus resulting in strong allelic imbalances at the corresponding marker loci and in stratification of the germplasm considered.

The durum wheat germplasm herein analysed appears well-differentiated and structured in a few, well-identified, main breeding groups. In particular, molecular, pedigree and published data regarding breeding and statistics of durum wheat in Italy pointed out the relative narrow genetic basis of materials cultivated in Italy during the first "breeding era" (until the mid-'70s), which was dominated by a few genotypes of Mediterranean origin with superior quality and adaptive characteristics over the mostly diverse local populations. In the following decades, the cultivated pool was enriched by a continuous flow of new and genetically diverse innovative materials (to a large extent different from the native and locally adapted Mediterranean germplasm), which broadened the genetic bases of the elite gene pool, as effectively shown by PCoA.

Conclusive remarks

The observation that a substantial amount of the total genetic variability present in the modern germplasm could be accounted for by alleles introduced with a limited number of founders is in line with the findings of Autrique et al. (1996), who reported a reduction of the genetic basis of modern durum wheat as compared to the native landrace gene pool locally cultivated. In that study, 85% of the total number of restriction fragments detected among improved varieties traced back to 11 ancestral accessions. In spring barley, 72% of the variation could be traced to alleles found in only 12 old accessions (Russell et al. 2000). Similar results were reported in other studies assessing the cultivated gene pools of barley and maize (Allard 1996, 1999) and were interpreted as the consequences of the purifying selection occurred over breeding cycles.

The modern elite durum germplasm has substantially retained the variability entered by a few outstanding and well-differentiated founders released through breeding cycles. More importantly, our results clearly indicate that the variability has increased over the last two decades. It should also be noticed that this recent broadening is an ongoing process.

Despite the limited amount of genetic variation that could be ascribed to the "rare allelic variants" in the elite gene pool surveyed herein, a noticeable number of different rare alleles were detected (32% of the total number of allelic variants), which indicates that a large number of plant introductions actually contributed to the genetic bases of this germplasm. Similar results were also

observed in the SSR-based survey of spring barley cultivated in Northern Europe (Russell et al. 2000).

During the '80s and the '90s, the introduction in the main breeding programs conducted in the Mediterranean countries of new elite and genetically diversified germplasm from CIMMYT and North America led to the release of a new generation of modern varieties adapted to the Mediterranean basin that mark new progresses as to yield potential, yield stability and semolina quality. An association mapping approach exploiting a detailed fingerprinting and phenotyping of the elite germplasm, combined with a retrospective analysis, should lead to the identification of genomic regions valuable as selection targets for agronomically and qualitatively important traits.

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