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Using AFLPs to determine phylogenetic relationships and genetic erosion in durum wheat cultivars released in Italy and Spain throughout the 20th century

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Abstract

Characterization of germplasm by DNA-markers provides a tool for precise germplasm identification and a quantitative assessment of genetic diversity. The genetic structure of the durum wheat germplasm grown in the Mediterranean basin varies largely from traditional landraces and cultivars characterized by a high versatility, to the modern varieties characterized by high yield potential, wide adaptation, and commercial end-use quality. The objective of this study was to assess the phylogenetic relationships among 24 durum wheat cultivars selected from relevant germplasm obtained at different periods in Italy and Spain, and to quantify the genetic erosion caused in durum wheat by breeding activities during the last century in these two countries.

Genetic similarity between cultivars was studied by AFLP markers through the calculation of the Dice's coefficient. The results showed a high degree of genetic similarity between the old Spanish cultivars and the collection of Italian cultivars, suggesting that wheat could have been introduced in the Iberian Peninsula via Italy. Genetic diversity estimates based on AFLP data confirmed the maintenance of genetic diversity with time since the values of Polymorphic Information Content were 0.27 for old cultivars (released before 1945), 0.28 for intermediate cultivars (released between 1950 and 1985) and 0.29 for modern cultivars (released between 1988 and 2000). These results indicate that genetic variability in Italian and Spanish durum wheat seems to have been maintained quite constant throughout the breeding process over the last century.

Keywords: AFLP marker; Genetic diversity; Durum wheat; Genetic distances; Molecular markers; Phylogenetic relationships

1. Introduction

Durum wheat (*Triticum turgidum* L. var. *durum*) is an important small grain cereal, mainly used for human consumption. Recently, this crop has been the subject of renewed interest, because of its valuable production and adaptation to low-rainfall, semiarid

Abbreviations: AFLPs, amplified fragments length polymorphism; CIMMYT, centro internacional de mejoramiento de maíz y trigo; ICARDA, international centre of agricultural research in the dry areas; MDS, multidimensional scaling; MI, marker index; PIC, polymorphism index content; UPGMA, unweighted pair-group method with the arithmetic averages.

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environments and its unique end products. More than half of the durum acreage lies in the Mediterranean basin, mainly in Italy, Spain, France, Greece, and the West Asia and North Africa (WANA) region, where this cereal has historically received special attention as a relevant crop (Belaid, 2000; Maccaferri et al., 2003).

It is widely believed that the genetic diversity of major crops, including durum wheat, has suffered an overall reduction with time, primarily as a consequence of domestication processes and, more recently, as a result of the recurrent use of adapted germplasm and the adoption of breeding schemes not favoring wide genetic recombination (Allard, 1996; Hoisington et al., 1999; Donini et al., 2000).

The genetic structure of the durum germplasm grown in the Mediterranean basin varies largely from traditional landraces and cultivars, characterized by a high versatility, to the modern varieties characterized by high yield potential, wide adaptation and technological quality (Bozzini et al., 1998). Whereas durum wheat breeding has been a traditional activity in Italy since the beginning of the last century, when Nazareno Strampelli initiated his crossing program (Maliani, 1979), the durum wheat variety structure in Spain has been mostly based on the introduction of foreign germplasm. Particularly, varieties derived from CIMMYT germplasm have been continuously introduced from the 1970s.

Molecular markers have provided a powerful approach to analyse genetic relationships among accessions in many crop species. Molecular markers are a useful complement to morphological and physiological characterization of cultivars because they are plentiful, independent of plant tissue or environmental effects, and allow cultivar identification very early in plant development (Manifesto et al., 2001). Molecular characterization of cultivars is also useful to evaluate potential genetic erosion, i.e., a reduction of genetic diversity along the breeding process. DNA-based markers are particularly useful in wheat and other crops with an apparent narrow genetic background.

Genetic diversity in the *Triticeae* has been explored using a range of molecular markers (reviewed in Gupta et al., 1999). Extensive information is available for rice (Ishii and McCouch, 2001; Prashanth et al., 2002), maize (Smith et al., 1997; Lu and Bernardo, 2001) and barley (Ellis et al., 1997). Among small grain cereals, barley and bread wheat adapted germplasm has been successfully investigated in detail with high-throughput, PCR-based molecular markers, such as Amplified Fragment Length Polymorphisms, AFLPs (Barrett and Kidwell, 1998; Manifesto et al., 2001) and Sequence Tagged Microsatellite Sites (STMSs or, more generally, SSRs; Prasad et al., 2000; Russell et al., 2000), while only recently similar PCR-based molecular surveys have focused on durum wheat (Soleimani et al., 2002). The efficiency of polymorphism detection by AFLP in wheat is high compared with other available marker systems (Soleimani et al., 2002; Tuberosa et al., 2002; Hazen et al., 2002; Almanza-Pinzon et al., 2003) since the AFLP technique combines the RFLP reliability with the power of PCR to amplify simultaneously many restriction fragments (Vos et al., 1995).

In this study, we present the molecular characterization, by using AFLP markers, of 24 durum wheat cultivars selected from relevant germplasm obtained at different periods in Italy and Spain. The objectives of this work were (i) to estimate the phylogenetic relationships between these Italian and Spanish cultivars and (ii) to quantify the genetic erosion caused during the last century in durum wheat by breeding activities in both Mediterranean countries.

2. Materials and methods

2.1. Plant material

Twenty-four durum wheat cultivars were selected to represent the germplasm grown in Italy and Spain during the last century. The plant material included 12 Italian and 12 Spanish durum wheat cultivars, from three different periods: old (obtained before 1945), intermediate (between 1950 and 1985) and modern (between 1988 and 2000). Table 1 lists the relevant information about the cultivars used. Plant material was cultivated in an environment-controlled chamber under 600 μmol photons m^{-2} s^{-1} of irradiance, 25 °C day/15 °C, constant relative humidity of 80% and photoperiod of 16 h/8 h. Plants were sampled at the five leaf stage and frozen at −80 °C.

2.2. DNA extraction

DNA was isolated using a modified cetyltrimethylammonium bromide (CTAB) method (Saghai-

Table 1
Description of the 24 Italian and Spanish durum wheat cultivars

Cultivar	Year of release	Registered pedigree	Origin
Italian			
Old			
Balilla F.	<1930	Not recorded	Not recorded
Carlojucci	1945	Russello/Forlani	Not recorded
S. Capelli	1930	Jennah/Khetifa	Strampelli
Razza 208	<1930	Not recorded	Not recorded
Intermediate			
Adamello	1985	Valforte/Selezione Turca	Isi. Sper. Cerealicoltura
Capeiti 8	1955	S. Capelli/Eiti	Stazione Granic., Sicilia
Creso	1974	CpB14 × (((Yt 54 × N10 × B) × Cp 63 2) × TC 603)	ENEA
Trinakria	1970	B 14/Capeiti 8	Inst. Agronom., Palermo
Modern			
Cirillo	1992	Carlojucci/Polesine//Creso/Montanari	Miliani genética
Flavio	1992	Latino/Cappelli	SIS
Simeto	1988	Capeiti 8/Valnova	Stazione Granic., Sicilia
Zenit	1992	Valriccardo × Vie	SPS Bologna
Spanish			
Old			
Blanco V.	<1930	Not recorded	Sevilla
Clarofino	<1930	Not recorded	Albacete
Pinet	<1930	Not recorded	Valencia
Rubio B.	<1930	Not recorded	Córdoba
Intermediate			
Bidi-17	1950	Not recorded	Algeria
Camacho	1975	Jerez 36 × Mex 246 × Lebrija	INIA España
Esquilache	1976	Not recorded	Semillas Agrícolas SA
Mexa	1980	GDOVZ469/3/Jo's//61130/LSL	CIMMYT-Mexico
Modern			
Ariesol	1992	Not recorded	Agrar Semillas
Astigi	1999	Not recorded	ASGROW Semillas
Boabdil	2000	Not recorded	IRTA/Semillas Fitó
Senadur	1995	Not recorded	SENASA

Maroof et al., 1984). For each cultivar, about 5 g of bulked leaf tissue collected from 20 plants was ground to a fine powder using liquid nitrogen and then suspended in 20 ml of extraction buffer (20 mM EDTA (pH 8.0), 100 mM Tris–HCl (pH 8.0), 1.5 M NaCl, 2% CTAB, and 1% β-mercaptoethanol). The suspension was mixed well, incubated at 60 °C for 45 min, followed by chloroform–isoamyl alcohol (24:1) extraction and precipitation with 2/3 of the volume of isopropanol at –20 °C for 1 h. The DNA was pelleted down by centrifugation at 12,000 rpm for 10 min and suspended in TE buffer (10 mM Tris-HCl — 1 mM EDTA (pH 8.0). The DNA was purified from RNA and proteins by standard measures (Sambrook et al., 1989) and DNA concentration was estimated by agarose-gel electrophoresis and staining with ethidium bromide.

2.3. AFLP analysis

Genomic DNA (250 ng) was digested to completion with 5 U each *Mse*I and *Pst*I restriction enzymes (Boehringer-Manheim, Germany). The double-digested DNA fragments were ligated to 5 pmol *Pst*I and 50 pmol *Mse*I adaptors (Vos et al., 1995). The adaptor-ligated DNA was diluted 10 times with TE buffer and subjected to pre-selective amplifications with *Mse*I adaptor +C and *Pst*I +A primers. A 25 μl reaction mixture containing 2.5 μl diluted DNA,

75 ng of each primer, 1× reaction buffer (10 mM Tris–HCl (pH 8.3), 50 mM KCl, and 1.5 mM $MgCl_2$), 1 U Taq DNA polymerase (Bangalore Genei, India) and 250 μM of dNTPs was subjected to 20 cycles of 94 °C for 30 s, 56 °C for 60 s, and 72 °C for 60 s in a thermal cycler (Genyus). The concentration of the amplified DNA was checked in 1.5% agarose gel and diluted 25 times in TE buffer.

Selective amplifications were performed with 1 μl of nondiluted preamplification product and 30 ng of each selective nonlabeled +3 primer, 1 × buffer (20 mM Tris–HCl (pH 8.4), 1.5 mM $MgCl_2$, and 50 mM KCl), 125 μM dNTPs, and 1.0 U *Taq* DNA polymerase. Selective amplification was carried out for one cycle at 94 °C for 30 s, 65 °C for 30 s, and 72 °C for 60 s, and the annealing temperature was then repeatedly lowered by 1 °C for each of the next nine cycles, followed by 23 cycles at 94 °C for 15 s, 56 °C for 30 s and 72 °C for 30 s. Amplified products were mixed with equal volume of formamide loading buffer (98% formamide, 10 mM EDTA, 0.1% bromophenol blue, 0.1% xylene cyanol), denatured at 90 °C for 3 min, and resolved on 6% denaturing polyacrylamide gels (acrylamide-bis-acrylamide (20:1), 7.5 M urea × TBE buffer) at 60 W constant power in 1 × TBE buffer running buffer for 3.75 h. Marker lanes contained 4 ng of 1 kb ladder (Gibco # 15615-016, Life Technologies) which exhibits 517-, 506-, 398-, 344-, 298-, 220-, 201-, 154-, 134-, and 75-bp fragments. AFLP bands were visualized by silver stain protocol (Bassam et al., 1991). The gel was visually scored while on the glass plate. The gels were dried overnight and scanned. To preserve the gel, it was agitated in 2% NaOH solution until it began to separate from the glass plate. The plate was then transferred into 3% acetic acid for 3 min, and then transferred into ddH_2O for 3 min. Fourteen-primer combinations used for selective amplifications are reported in Table 2. The combinations of primer were (code): *P1M2*, *P1M3*, *P1M4*, *P2M1*, *P2M2*, *P2M3*, *P2M4*, *P2M5*, *P2M6*, *P3M1*, *P3M2*, *P3M4*, *P3M5*, *P3M6*.

Table 2
Oligonucleotide sequences used in AFLP analysis

Primer	Code	Sequence
*Pst*ACA	P1	GACTGCGTACATGCAG*ACA*
Pst*ACC*	P2	GACTGCGTACATGCAGACC
Pst*AAC*	P3	GACTGCGTACATGCAGAAC
*Pst*AAG	P4	GACTGCGTACATGCAGAAG
*Pst*ACG	P5	GACTGCGTACATGCAGACG
*Pst*ACT	P6	GACTGCGTACATGCAGACT
*Mse*CAA	M1	GATGAGTCCTGAGTAACAA
Mse*CAC*	M2	GATGAGTCCTGAGTAACAC
*Mse*CAG	M3	GATGAGTCCTGAGTAACAG
*Mse*CAT	M4	GATGAGTCCTGAGTAACAT
*Mse*CTG	M5	GATGAGTCCTGAGTAACTG
*Mse*CCA	M6	GATGAGTCCTGAGTAACCA

All sequences are written 5′–3′.

2.4. Data acquisition and analysis

For each primer pair, the number of polymorphic and monomorphic bands was determined; however, monomorphic bands were excluded from data analyses. Bands clearly visible in at least one cultivar were scored (1 for present, 0 for absent) and entered into a data matrix. Fragments size was estimated by interpolation from the migration distance of marker fragments. Percentage of polymorphism was calculated as the proportion of polymorphic bands over the total number of bands. Variability for each locus was measured using the polymorphism index content (PIC) (Anderson et al., 1993).

$$\mathrm{PIC} = 1 - \Sigma p_i^2$$

where p_i is the frequency of the i^{th} allele.

The polymorphic information content (PIC) was calculated for each primer combination (Table 3). The marker index was calculated for each AFLP primer combination as MI = PIC × $\eta\beta$, where PIC is the mean PIC value, η the number of bands, and β is the proportion of polymorphic bands (Table 3) (Powell et al., 1996).

For the AFLP analysis, each polymorphic fragment was scored as a locus with two allelic classes. The maximum PIC value of an AFLP locus is 0.5 due to the codominant inheritance of this type of molecular marker. Differences in genetic diversity between the groups of cultivars released at the three different periods during the last century were evaluated by analysis of variance. PIC values were then calculated for each locus in every group of cultivars.

Genetic relationships among cultivars were calculated with the Dice's coefficient (Dice, 1945) using NTSYS-pc version 2.0 software (Rohlf, 2000). The resulting similarity matrix was first subjected to

Table 3
Characterization of the degree of polymorphism and quality of AFLP data generated with 14 primers combination

PC[a]	Total number of bands (*n*)	Polymorphic bands		PIC (S.D.)[b]	MI[c]	Missing values (%)[d]
		n	%			
P1M2	119	22	18.49	0.37 (0.13)	8.14	3
P1M3	70	9	12.86	0.41 (0.07)	3.69	1
P1M4	82	14	17.08	0.30 (0.16)	4.20	2
P2M1	90	10	11.11	0.29 (0.21)	2.90	1
P2M2	83	12	14.46	0.31 (0.16)	3.72	1
P2M3	55	7	12.73	0.34 (0.10)	2.38	1
P2M4	89	12	13.48	0.35 (0.09)	4.20	2
P2M5	100	16	16.00	0.34 (0.17)	5.44	2
P2M6	86	12	8.14	0.33 (0.14)	3.96	2
P3M1	65	6	9.23	0.30 (0.23)	1.80	1
P3M2	83	12	14.46	0.41 (0.13)	4.92	1
P3M4	86	17	19.77	0.32 (0.16)	5.44	1
P3M5	92	20	21.74	0.23 (0.22)	4.60	3
P3M6	107	17	15.89	0.39 (0.15)	6.63	2
Mean	86.21	13.29	14.67	0.34 (0.15)	4.43	1.64
Total	1207	186			8.14	

[a] PC: primer combination.
[b] PIC: mean PIC value observed for AFLPs of the particular PC; S.D.: standard deviation.
[c] MI: marker index.
[d] Proportion of missing values based on all data points.

cluster analysis by the unweighted pair-group method with the arithmetic averages (UPGMA) and then with a multidimensional scaling analysis (MDS) (Kruskal, 1964), an ordination technique that reveals patterns of relatedness within a matrix by assigning Cartesian coordinates in multi-dimensional space to each cultivar, again using NTSYS-pc (Rohlf, 2000). Coordinates obtained were used to create scatterplots and calculate pairwase Euclidean distance estimates between cultivars.

3. Results and discussion

3.1. Band scoring

Twelve different primers (Table 2) were used in fourteen combinations to generate AFLP fingerprintings. A total of 1207 AFLP bands were identified in the study, of which 186 were polymorphic with clear and reliable reading. The number of polymorphic bands ranged from 6 to 22 per gel with an average of 13.29 ± 6.7 per primer combination. The percentage of polymorphism ranged from 8.14% (in the combination *P-ACC*, *M-CCA*) to 21.74% (in the combination *P-AAC*, *M-CTG*) (Table 3). The marker index per primer combination varied from 1.80 to 8.14 with an average of 4.43 (Table 3). In addition, the proportion of missing values had no influence on these quality parameters. PIC values and marker indices were almost identical to those published by Bohn et al. (1999) and Manifesto et al. (2001) in wheat.

In our study, the polymorphic bands found in old, intermediate and modern cultivars were 29, 39 and 41, respectively, for the Spanish cultivars, and 42, 34 and 32 for the Italian cultivars, showing a higher degree of genetic similarity in the old Spanish cultivars than in the Italian ones. Moreover, Italian modern cultivars appear as more similar between them than the corresponding Spanish modern cultivars.

3.2. Phylogenetic relationships

To evaluate the phylogenetic relationships between Italian and Spanish cultivars a dendrogram based in the 186 loci AFLPs was constructed by UPGMA (Fig. 1). The Dice's coefficient of similarity among cultivars based on these 186 polymorphic AFLP loci showed a normal distribution, with an average of 0.72 ± 0.15. Similarity coefficients ranged from

0.52 (cultivars Ariesol and Simeto) to 0.97 (cultivars Rubio de Belalcazar and Blanco Verdeal).

The first bifurcation in Fig. 1 clearly separates a cluster with Italian cultivars plus old Spanish cultivars from a cluster with intermediate and modern Spanish cultivars. Thus, the old Spanish cultivars tend to group with the cluster of the Italian cultivars, which seems to indicate a possible common geographical origin, as discussed later.

Information available indicates that the durum wheat germplasm usually grown in Italy until 1970 seems to be structured on a few well-identified breeding groups, with a relatively narrow genetic basis, which was dominated by a few genotypes of Mediterranean origin, with superior quality and adaptive characteristics over the mostly diverse local populations (Bozzini et al., 1998). In effect, according to the pedigree information (Table 1), a clear genetic relationship was observed within the Italian cultivars. Thus, at the center of the dendrogram (Fig. 1) appears Senatore Capelli, considered as the most important historical source for Italian germplasm, and two intermediates cultivars, Capeiti (also another important source of germplasm derived from S. Capelli) and Trinakria (genotype, in turn, derived of Capeiti) (Table 1). In other subcluster appear Simeto and Adamello, indicating a common origin from the section Valnova/Valforte germplasm, two cultivars genetically very close released in Italy in 1975 and 1980, respectively (Maccaferri et al., in press), and that have contributed to create a notable biodiversity in the modern Italian durum wheat.

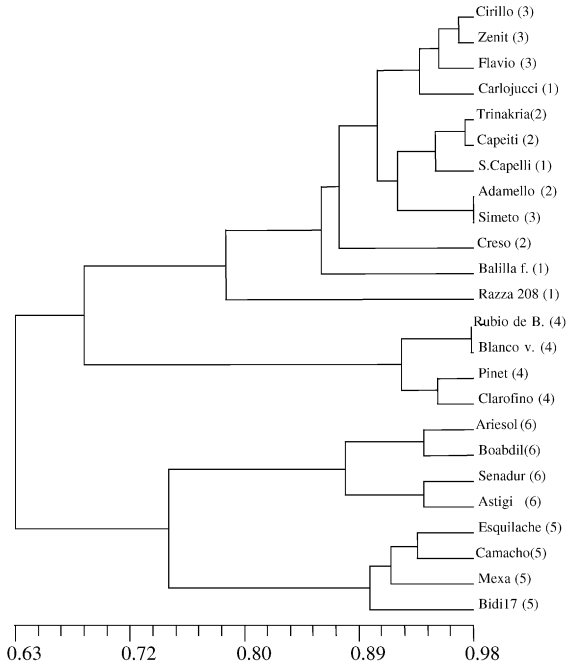

Fig. 1. Dendrogram of 24 cultivars of durum wheat from Italy and Spain released in the last century based on 186 AFLP fragments. (1) Old Italian; (2) intermediate Italian; (3) modern Italian; (4) old Spanish; (5) intermediate Spanish; (6) modern Spanish.

Creso, a cultivar released in 1974 and derived from a cross between S. Capelli and one cultivar proceeding from CIMMYT, grouped out of the mentioned cluster, but very close to the central subcluster derived from S. Capelli (Fig. 1) To a considerable distance of the remainder genotypes, appear Balilla Falso and Razza 208, two very ancient Italian cultivars, about which it was not possible to find any additional information, and which do not seem to have significantly contributed to the foundation of the modern elite Italian germplasm used in this study.

Finally, in the upper part of the dendrogram, appear three modern Italian cultivars (Cirillo, Zenit y Flavio), along with the older cultivar Carlojucci, because Cirillo is a genotype that directly proceeds from this last (see Table 1). This seems to indicate that these modern cultivars derive from similar common ancestors, but with the introgression of foreign germplasm, increasing thus the diversity in recent Italian durum wheat genetic background.

With regard to the Spanish cultivars, the dendrogram (Fig. 1) reveals a clear organization in subgroups related to the period of cultivar release. Thus, the four old cultivars (Rubio de Belalcazar, Blanco Verdeal, Pinet and Clarofino) appear in a subgroup clearly separated from the intermediate and modern ones. Moreover, the two cultivars Rubio de Belalcazar and Blanco Verdeal are located very closely, probably due to the fact that they were collected in two very close geographical areas (Spanish provinces of Córdoba and Sevilla, respectively). In turn, the dendrogram also reveals the similarity of the cultivars Pinet and Clarofino (Fig. 1), again because they have a proximate geographical origin (Spanish provinces of Valencia and Albacete, respectively).

The Spanish intermediate and modern cultivars group in the same section of the dendrogram

(Fig. 1), being placed more distant than the older genotypes, which tend to group more proximate to the Italian cultivars, as indicated above. The dendrogram, also clearly separate the intermediate cultivars from the modern ones (Fig. 1).

Actually, the Spanish intermediate cultivars used in this study include directly or indirectly germplasm from CIMMYT. Thus, Mexa is a selection from the CIMMYT genotype Mexicali 75, widely extended and used around the world. On the other hand, during the 1970s, CIMMYT used largely Bidi-17 for its crossing program. Bidi-17 is a selection from Bidi or Ble Gounod, a cultivar of Algerian origin that led to the release in Italy and Spain of a small number of successful varieties, which retained a consistent portion of the genetic structure of the CIMMYT parent. Moreover Camacho has on its pedigree a Mexican line. The present results demonstrate that also Esquilache had CIMMYT germplasm on its pedigree. The dendrogram also indicates that modern Spanish varieties retain a considerable portion of CIMMYT germplasm, but somehow different from the existing in intermediate cultivars. This result suggests that new sources of alleles have been used in the crosses that led to the more recently released varieties in Spain, but it is not possible to ensure whether these crosses were made at CIMMYT or in the own country.

The most relevant information obtained from this AFLPs analysis concerns the different breeding strategies followed in Italy and Spain during the 20th century. While in Italy ancient local cultivars seem to have been incorporated and their alleles recombined within the process of genetic improvement, old Spanish cultivars (released before 1945) do not seem to have been included within the genetic pool used for breeding modern varieties. This was probably due to the limited tradition of durum wheat breeding in Spain between the years 1940 and 1970, and highlights the primary role that germplasm developed at CIMMYT has played in the varietal structure of durum wheat in Spain.

The results of the dendrogram analysis were corroborated by means of a multidimensional scaling analysis, MDS (Kruskal, 1964), an ordination technique that reveals patterns of relatedness within a matrix by assigning Cartesian coordinates in a multi-dimensional space to each genotype. MDS complements cluster analysis by providing spatial representation of relative genetic distances among genotypes (Fig. 2). Although higher order MDS minimized stress, three-dimensional MDS provided an acceptable compromise between fit to the genetic diversity estimate matrix (stress 1 = 0.03059) and visual interpretation. Thus, visual interpretation of MDS (Fig. 2) confirms

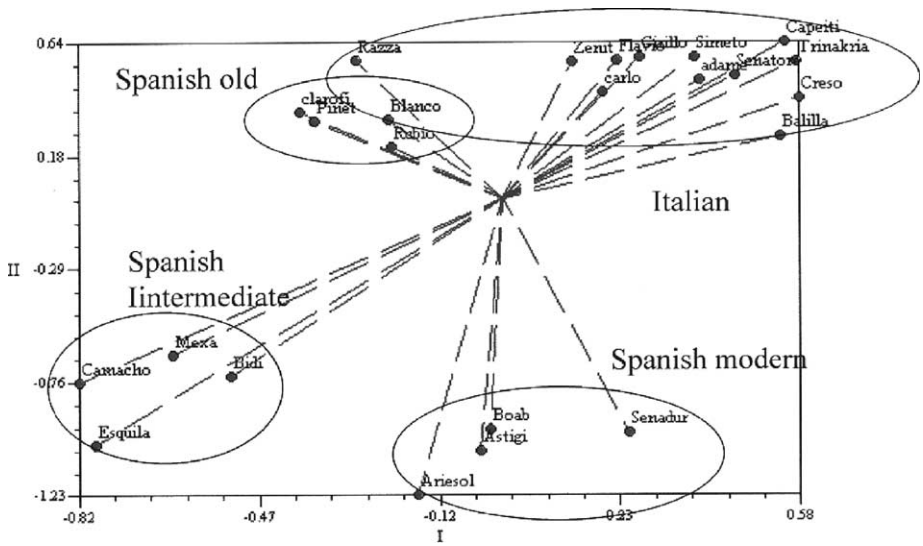

Fig. 2. Multidimensional scaling of 24 durum wheat cultivars representative of the durum germplasm relevant for the Italy and Spain. Diversity estimates are based on 186 AFLP markers.

the genetic relationships obtained in the dendrogram analysis, showing again a high degree of genetic similarity between the old Spanish cultivars and the set of Italian ones. This reinforces, as suggested before, the hypothesis that some Spanish durum wheat germplasm may have been introduced in the Iberian Peninsula via Italy in the past, also indicating a common geographical origin for them.

In this way, a recent study on the origin of cultivated wheat (Feldman, 2001) reveals that wheat expanded from the Fertile Crescent to Turkey, Greece and Italy about 10,000 years before present, passing from Italy to the Iberian Peninsula and North of Africa about 1000 years later. The genetic proximity encountered in this study between the Spanish landraces and the Italian genotypes tends to support this route as one of the ways of expansion of durum wheat to the Iberian Peninsula.

3.3. Genetic erosion through breeding

One goal of this study was to investigate the usefulness of AFLP fingerprinting for estimating the levels of genetic diversity in a historical series of Italian and Spanish durum wheat cultivars with an apparent narrow genetic variability (Dice's coefficient = 0.72). It is generally believed that modern breeding practices have led to a significant decrease of genetic diversity in modern varieties (Vellvé, 1993). There is concern that this erosion of the genetic variability might result in reduction of the plasticity of crops to respond to changes in climate, pathogen populations, agricultural practices, or quality requirements (Manifesto et al., 2001).

In the entire collection of genotypes, PIC values obtained ranged from 0.23 to 0.41, with an average value of 0.34 ± 0.15. Between times of release, the diversity values in the three different periods ranged from 0.27 to 0.29 (Table 4) without statistical differences. This absence of significant variation in the polymorphism index obtained in the three periods studied, indicated that the overall genetic diversity of durum wheat remained quite constant throughout genetic improvement occurring during the 20th century, at least across these 24 cultivars representative of Italian and Spanish germplasm. Thus, apparently, the extent of variability available to the growers today is similar to the extent of variability existing in the cultivars released before 1945, with a little increase with time, but without significant differences between periods of release. Similar results have been found in a study with spring wheat cultivars released in UK between 1934 and 1994 (Donini et al., 2000) and with bread wheat genotypes released in Argentina between 1932 and 1995 (Manifesto et al., 2001). Souka et al. (1994) also found similar results examining genetic diversity in spring wheat cultivars grown in the Yaqui Valley of Mexico and in the Punjab region of Pakistan. These results are probably due to the fact that these regions took advantage of the semi dwarf genotypes released during the Green Revolution in the early 1960s, which probably contributed to increase the genetic diversity in the local germplasm. In fact, a recent molecular study by Parker et al. (2002) on the impact of introduction of a dwarfing gene in an Australian breeding program showed an enhancement of genetic diversity.

Table 4
Comparison of average PIC values in each period ($P < 0.05$)

	Period of cultivar release	N[a]	Average PIC values
Old	<1945	8	0.27 ± 0.02 A
Intermediate	1950–1985	8	0.28 ± 0.02 A
Modern	1988–2000	8	0.29 ± 0.02 A

[a] N: number of cultivars included in each category.

It is possible that this also happened with germplasm used in the present study, where the Spanish and Italian cultivars retained genetic diversity during the last century. Actually, the older genotypes were mainly grown in a very local and restricted geographical area, making transport to distant zones from their local origin difficult, with the exception of population movements. The Green Revolution and the foundation of international centres (such as CIMMYT and ICARDA) have enormously promoted shuttle breeding and the intensive interchange of germplasm around the world. This could be the reason why cultivated durum wheat globally presently has a common genetic background. In fact, the pedigree of the Italian genotypes used in this study shows high influence from Capeiti 8, a genotype derived from a cross between S. Capelli and Eiti (a Palestinian cultivar, which has very different characteristics to the Italian founders, such as reduced plant height). The wide use of S. Cappelli during the first half of the 20th

century in the Italian (and world-wide) breeding programs has been reported (Bozzini et al., 1998). In addition, the second important ancestor (Eiti), has been also included by Autrique et al. (1996) among the five ancestral lines presented in all pedigrees of the advanced materials developed by the CIMMYT/ICARDA breeding program.

Results of the present study indicate that extent of genetic variability in Italian and Spanish durum wheat seems to have remained quite constant over the last century. This constancy should be considered of qualitative relevance, as it indicates that cultivated pool was enriched by material different from the native and locally adapted Mediterranean germplasm, which resulted in a consistent broadening of the genetic background in these countries.

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References

Almanza-Pinzon, M.I., Khairallah, M., Fox, P.N., Warburton, M.L., 2003. Comparison of molecular markers and coefficients of parentage for the analysis among spring bread wheat accessions. Euphytica 130, 77–86.

Allard, R.W., 1996. Genetic basis of the evolution of adaptedness in plants. Euphytica 92, 1–11.

Anderson, J.A., Churchill, J.E., Autrique, S.D., Tanksley, S., Sorrells, M.E., 1993. Optimizing parental selection for genetic linkage maps. Genome 36, 181–188.

Autrique, E., Nachit, M.M., Monneveux, P., Tanksley, S.D., Sorrells, M.E., 1996. Genetic diversity in durum wheat based on RFLPs, morphophysiological traits, and coefficient of parentage. Crop Sci. 36, 735–742.

Barrett, B.A., Kidwell, K.K., 1998. AFLP-based genetic diversity assessment among wheat cultivars from the Pacific Northwest. Crop Sci. 38, 1261–1271.

Bassam, B.J., Caetano-Anolles, G., Greshoff, P.M., 1991. Fast and sensitive silver staining of DNA in polyacrylamide gel. Anal. Biochem. 196, 80–83.

Belaid, A., 2000. Durum wheat in WANA: production, trade, and gains from technological change.In: Di Fonzo, N., Kaan, F., Nachit, M. (Eds.), 2000. Durum Wheat Improvement in the Mediterranean Region: New challenges, vol. 40. Options Méditerranéennes, CIHEAM, Zaragoza, pp. 35–50.

Bohn, M., Friedrich, U.H., Melchinger, A.E., 1999. Genetic similarities among winter cultivars determined on the basis of RFLPs, AFLPs, and SSRs and their use for predicting progeny variance. Crop Sci. 39, 222–237.

Bozzini, A., Corazza, L., D'Egidio, M.G., Di Fonzo, N., La Fiandra, D., Pogna, N.E., Poma, I., 1998. Durum wheat (*Triticum turgidum* spp. durum). In: Scarascia Mugnozza, G.T., Pagnotta, M.A. (Eds.), Italian Contribution to Plant Genetics and Breeding, Viterbo, Italy, pp. 181–194.

Dice, L.R., 1945. Measurements of the amount of ecologic association between species. Ecology 26, 297–302.

Donini, P., Law, J.R., Koebner, R.M.D., Reeves, J.C., Cooke, R.J., 2000. Temporal trends in the diversity of UK wheats. Theor. Appl. Genet. 100, 912–917.

Ellis, R.P., Mc Nicol, J., Baird, E., Booth, A., Lawrence, P., Thomas, B., Powell, W., 1997. The use of AFLP to examine genetic relatedness in barley. Mol. Breed. 3, 359–369.

Feldman, M., 2001. Origin of cultivated wheat.In: Bonjean, A.P., Angus, W.J. (Eds.), 2001. The World Wheat Book. A History of Wheat Breeding, Lavoisier Publishing, Paris, pp. 3–56.

Gupta, P.K., Varshney, R.K., Sharma, P.C., Ramesh, B., 1999. Molecular markers and their applications in wheat breeding. Plant Breed. 118, 369–390.

Hazen, S.P., Leroy, P., Ward, R.W., 2002. AFLP in Triticum aestivum L. patterns of genetic diversity and genome distribution. Euphytica 125, 89–102.

Hoisington, D., Khairallah, M., Reeves, T., Ribaut, J.M., Skovmand, B., Taba, S., Warburton, M., 1999. Plant genetic resources: what can they contribute toward increased crop productivity? Proc. Natl. Acad. Sci. U.S.A. 96, 5937–5943.

Ishii, T., McCouch, S.R., 2001. Microsatellites and microsynteny in the chloroplast genomes of *Oryza* and other Gramineae species. Theor. Appl. Genet. 100, 1257–1266.

Kruskal, J.B., 1964. Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. Psychometrika 29, 1–27.

Lu, H., Bernardo, R., 2001. Molecular marker diversity among current and historical maize inbreds. Theor. Appl. Genet. 103, 613–617.

Maccaferri, M., Sanguineti, M.C., Donini, P., Tuberosa, R., 2003. Microsatellite analysis reveals a progressive widening of the genetic basis in the elite durum wheat germplasm. Theor. Appl. Genet. 107, 783–797.

Maccaferri, M., Sanguineti, M.C., Donini, P., Porceddu, E., Tuberosa, R. A retrospective analysis of genetic diversity in durum wheat elite germplasm based on microsatelite analysis: a case study. In: Royo, C., Nachit, M.N., Di Fonzo, N., Araus, J.L., Pfeiffer, W.H., Slafer, G.A. (Eds.), Durum Wheat Breeding: Current Approaches and Future Strategies. Haworth Press, New York, in press.

Manifesto, M.M., Schlatter, A.S., Hopp, H.E., Suarez, E.Y., Dubcovky, J., 2001. Quantitative evaluation of genetic diversity germplasm using molecular markers. Crop Sci. 41, 682–690.

Maliani, C., 1979. Nazareno Strampelli, a fore-runner in green revolution. Genet. Agr. 33, 1–4.

Parker, G.D., Fox, P.N., Langridge, P., Chalmers, K., Whan, B., Ganter, P.F., 2002. Genetic diversity within Australian wheat breeding programs based on molecular and pedigree data. Euphytica 124, 293–306.

Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S., Rafalsky, A., 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol. Breed. 2, 225–238.

Prasad, M., Varshney, R.K., Roy, J.K., Balyan, H.S., Gupta, P.K., 2000. The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. Theor. Appl. Genet. 100, 584–592.

Prashanth, S.R., Parani, M., Mohanty, B.P., Talame, V., Tuberosa, R., Parida, A., 2002. Genetic diversity in cultivars and landraces of *Oryza sativa* subsp. *indica* as revealed by AFLP markers. Genome 45, 451–459.

Rohlf, F.J., 2000. NTSYS-pc numerical taxonomy and multivariate system (v.2.1). User guide. Exeter Software, Setauket, New York.

Russell, J.R., Ellis, R.P., Thomas, W.T.B., Waugh, R., Provan, J., Booth, A., Fuller, J., Lawrence, P., Young, G., Powell, W., 2000. A retrospective analysis of spring barley germplasm development from "foundation genotypes" to currently successful cultivars. Mol. Breed. 6, 553–568.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning: a Laboratory Manual, second ed. Cold spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A., Allard, R.W., 1984. Ribosomal spacer length polymorphism in barley: mendelian inheritance, chromosomal location and population dynamics. Proc. Natl. Acad. Sci. U.S.A. 83, 1757–1761.

Smith, J.S.C., Chin, E.C.L., Shu, H., Smith, O.S., Wall, S.J., Senior, M.L., Mitchell, S.E., Kresovich, S., Ziegle, J., 1997. An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.). Theor. Appl. Genet. 95, 163–173.

Soleimani, V.D., Baum, B.R., Jonson, D.A., 2002. AFLP and pedigree-based genetic diversity estimates in modern cultivars of durum wheat [*Triticum turgidum* L. subsp *durum* (Desf.) Husn.]. Theor. Appl. Genet. 104, 350–357.

Souka, E.P., Fox, P.N., Byerlee, D., Skovmand, B., 1994. Spring wheat diversity in two developing countries. Crop Sci. 34, 774–783.

Tuberosa, R., Gill, S., Bikram, Quarrie, S., 2002. Cereal genomics: ushering in a brave new world. Plant. Mol. Biol. 48, 445–449.

Vellvé, R., 1993. The decline of diversity in European agriculture. Ecologist 23, 64–69.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Hornes, M., Fritjers, A., Pot, J., Peleman, J., Kuiper, M., Zabeau, M., 1995. AFLP — a new technique for DNA-fingerprinting. Nucleic Acids Res. 23, 4407–4414.