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Molecular characterization of emmer (*Triticum dicoccon* Schrank) Italian landraces

G. Barcaccia[1], L. Molinari[2], O. Porfiri[2] and F. Veronesi[2,*]
[1]*Dipartimento di Agronomia Ambientale e Produzioni Vegetali, Università degli Studi di Padova, AGRIPOLIS, Legnaro, Padova 35020, Italy;* [2]*Dipartimento di Biologia Vegetale e Biotecnologie Agrea Ambientali, Università degli Studi di Perugia, Borgo XX Giugno 74, 06121 Perugia, Italy;* [*]*Author for correspondence (e-mail: veronesi@unipg.it; phone: +39 075 585 6207; fax: +39 075 585 6224)*

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Abstract

Emmer (*Triticum dicoccon* Schrank, $2n = 4x = 28$) is a hulled wheat species [more] widely spread in the Mediterranean basin. In Italy it survives as a crop in a few marginal areas and peculiar ecological niches in different regions of central and southern Italy. A renewed interest has occurred during the last decade toward local varieties belonging to this species. As a matter of fact, local varieties have the highest genetic variation and adaptation to the natural and anthropological environment from where they originated. Results on the genetic diversity within and relationships among 11 Italian local varieties of emmer as assessed with 17 RAPD marker loci are here reported. The proportion of the among-local variety genetic diversity was as high as 48% ($G_{ST} = 0.479$). Thus, about 52% of the total variation was within population. Local varieties of emmer proved to be formed by a variable number of lines genetically distinguishable from each other, and the vast majority of individuals over populations proved to be different multilocus genotypes. Landraces of emmer from central and southern Italy showed distinctive molecular traits. In particular, local varieties classified as «Central Italy» types were characterized by a common set of RAPD marker alleles and proved to be distinguishable from both the «Southern Italy» and the «Garfagnana» accessions. The overall results confirm the high variability that can be found within landrace populations, underlining the values of landraces as an irreplaceable bank of genetically diversified and highly co-adapted genotypes. Information for an appropriate *in situ* conservation and management of this valuable source of emmer germplasm is discussed.

Introduction

The hulled wheats include three cultivated species with different ploidy levels: einkorn (*Triticum monococcum* L., $2n = 2x = 14$), emmer (*Triticum dicoccon* Schrank, $2n = 4x = 28$) and spelt (*Triticum spelta* L., $2n = 6x = 42$) (Szabo and Hammer 1996). They are called «neglected» and nowadays are still growing spontaneously in marginal areas around the origin and diversification centres. So far their major role is mainly that of a source of genes controlling important agronomic traits useful for the improvement of free-threshing wheats.

Emmer is more widely spread in the Mediterranean basin. In Italy it survives as a crop in a few marginal areas and peculiar ecological niches in different regions of central and southern Italy (Porfiri et al. 1998a). A renewed interest has occurred during the last decade toward local varieties belonging to this species. The reasons are various: the need for crop diversification; the higher need for genetic resources conservation; the increasing concern for the environmental and healthy aspects deriving from crop cultivation systems; the rediscovery of typical, local food products (Porfiri et al. 1998b).

Local varieties have the highest genetic variation and adaptation to the natural and anthropological environment in which they originated. They represent

an irreplaceable bank of highly co-adapted genotypes. Knowledge of genetic diversity within as well as genetic relatedness among populations from different geographic areas is expected to have a significant impact on the conservation and utilization programs of emmer germplasm. Despite this, the genetic characterization of local varieties as a key tool for their exploitation has been largely ignored until very recently. Information obtainable by surveying both qualitative and quantitative morphological plant and seed traits of existing local varieties may be useful in maintaining their genetic variability and preserving them from genetic erosion.

In the last few years, a number of collections of *T. dicoccon*, gathered by different research institutions of Italy and other Mediterranean countries, have been evaluated for morpho-phenological traits, qualitative and agronomic parameters (Castagna et al. 1996; Piergiovanni et al. 1996; D'Antuono and Minelli 1998; Cubadda and Marconi 1996; Laghetti et al. 1998; Porfiri et al. 1998c). These collections showed a large variability for all evaluated characters. Based on morpho-phenological data and geographic area of cultivation, the emmer materials were classified into three group types: «Garfagnana» (winter types, large spike with or without awns, large kernel with floury fracture); «Central Italy» (spring types, small spike with awns, medium-small kernel usually with vitreous fracture); «Southern Italy» (winter types, large spike with long awns, large kernel with both floury and vitreous fracture) (D'Antuono and Pavoni 1993; Porfiri et al. 1998a).

The potential of molecular markers as a method for monitoring and characterizing genetic variability is known. As a matter of fact, the plant DNA polymorphism assays are also powerful tools for investigating germplasm resources. These techniques include not only restriction fragment length polymorphism (RFLP) markers, but also PCR-based molecular markers. In particular, random amplified polymorphic DNA (RAPD) markers (Williams et al. 1990) assay subsets of the total amount of the genetic variation by detecting multiple loci randomly distributed in the genome: polymorphisms result from DNA sequence variation at primer binding sites and from DNA length differences between primer binding sites.

Results on the genetic diversity within and relationships among Italian local varieties of emmer as assessed with RAPD markers are reported here. Information for an appropriate *in situ* conservation and management of this valuable source of emmer germplasm is discussed.

Materials and methods

Plant material

Eleven local varieties of emmer (*T. dicoccon*) from central and southern Italy were collected within a research project financed by ARSSA (Agricultural Extension Service, Abruzzo Region, Italy). Two local varieties of both common wheat (*Triticum aestivum* L.) and durum wheat (*Triticum durum* Desf.) were used as reference standards. Each population was represented by 10 individuals from as many kernels randomly sampled in different spikes. Genetic diversity and relationships within and among all accessions were investigated with RAPD markers using 10-mer primers. Detailed information on germplasm stocks including collection sites and morpho-phenological traits are reported in Table 1.

Genomic DNA isolation

Approximately 0.5 to 0.75 g of leaf tissues were collected from healthy plants and frozen in liquid nitrogen. Total genomic DNA from leaf samples was isolated according to the protocol described by Barcaccia and Rosellini (1996). The DNA pellet was washed twice with 70% ethanol, dried and redissolved in 100 μL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The concentration of DNA samples was determined by optical density reading (DU650 spectrophotometer, Beckman) at 260 nm (1 OD = 50 μg/mL) and the purity calculated by the OD_{260}/OD_{280} ratio and by the OD_{210}–OD_{310} pattern (Sambrook et al. 1989). An aliquot of genomic DNA was also assayed by electrophoresis on 1% agarose gels.

RAPD markers

PCR parameters and gel electrophoresis in RAPD analysis were those described by Barcaccia (1994), with some changes. Briefly, reactions occurred in a 25-μL volume which included 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$ with 300 μM each of dCTP, dGTP, dATP and dTTP, 0.8 μM of a single primer, 1 U of *Taq* DNA polymerase (Pharmacia Biotech) and 30 ng of genomic DNA. Amplification reactions were performed in a Omnigene Thermal Cycler (Hybaid) under the following conditions: an initial denaturation at 95 °C for 5 min followed by 40 cycles of 45 s at 94 °C, 30 s at 36 °C, 1 min at 72 °C and a final step at 72 °C for 10 min. The rates of temperature change adopted for heating and cooling were +1 °C/2.9 s and

Table 1. Information on collection sites and morphophenological traits of emmer and wheat accessions as evaluated in experimental field in central Italy.

Accessions	Species	Collection site	Altitude m (a.s.l.)	Group type[1]	Growth habit	Plant type[2]	Spike characteristics		
							Colour	Awns	Size[3]
ERSA≠1	*T. dicoccon*	Torano Nuovo (TE)	237	"Central Italy"	Spring	Semi-erect	White	Present	Small
ERSA≠2	*T. dicoccon*	Guardiagrele (CH)	576	"Central Italy"	Spring	Semi-erect	White + red	Present	Small
ERSA≠3	*T. dicoccon*	Guardiagrele (CH)	576	"Southern Italy"	Winter	Semi-erect	White	Present	Large
ERSA≠4	*T. dicoccon*	Penne (PE)	438	"Southern Italy"	Winter	Semi-erect	White + red	Present	Large
ERSA≠5	*T. dicoccon*	Penne (PE)	438	"Central Italy"	Spring	Semi-erect	White	Present	Small
ERSA≠6	*T. dicoccon*	Civitaluparella (CH)	903	"Southern Italy"	Winter	Semi-erect	White	Present	Small
ERSA≠7	*T. dicoccon*	Montereale (AQ)	945	"Central Italy"	Spring	Semi-erect	White	Present	Small
ERSA≠8	*T. dicoccon*	Caporciano (AQ)	836	"Garfagnana"	Winter	Semi-erect	White	Pres/absent	Medium
LIVE≠105	*T. dicoccon*	Torano Nuova (TE)	237	"Southern Italy"	Winter	Semi-erect	White	Present	Large
AMAT≠107	*T. dicoccon*	Amatrice (RI)	955	"Central Italy"	Spring	Semi-erect	White	Present	Small
CAGN≠114	*T. dicoccon*	Castelvecchio (AQ)	1000	"Central Italy"	Spring	Semi-erect	Red	Present	Small
Belvedere	*T. aestivum*	Liscia (CH)	740	–	–	–	–	–	–
Frassinese	*T. aestivum*	Carunchio (CH)	714	–	–	Erect	White	Pres/absent	Medium
La Cappella	*T. durum*	Carunchio (CH)	714	–	Spring	Semi-prostrate	White	Pres (black)	Medium
Marzuolo	*T. durum*	Montenerodomo (CH)	1160	–	Spring	Semi-erect	White	Pres (white)	Medium

1. Classification of local varieties according to D'Antuono and Pavoni (1993), Porfiri et al. (1998c).
2. Scored as: very prostrate; prostrate; semi-prostrate; semi-erect; erect, according to U.P.O.V (1974).
3. Small $<$ 5 cm; Medium = 5–8 cm; Large $>$ 8 cm.

−1 °C/2.4 s, respectively. Polymerized genomic fragments were separated by electrophoresis in 1.5% agarose gels run with 1× TBE buffer. Photographs were taken of the ethidium bromide-stained gels visualized by UV light illumination.

The sequences (5′-3′) of the oligonucleotide primers (Operon Technologies, Inc.) used are the following: OPB-07=GGTGACGCAG, OPB-17=AGGGAACGAG, OPC-04=CCGCATCTAC and OPC-15=GACGGATCAG. These 10-*mers* were considered the best ones among a set of 32 primers analyzed in preliminary experiments on the basis of their ability to find homologous binding sites and to give DNA fingerprints with a reliable number of strong amplification products, regardless of the number of polymorphic bands, to avoid any bias due to the specific subsample of emmer DNA templates used (Barcaccia et al. 1998).

Data analysis

RAPD markers were scored as present (1) or absent (0) over all DNA samples of both emmer and wheat accessions. Data were recorded as a binary matrix by assigning the molecular weight to each monomorphic and polymorphic marker identified by comparing sample lanes with known DNA ladders.

Different measures of genetic variability were used to estimate the levels of polymorphism within and between different accessions. The average RAPD marker allele frequency (p_i) for each primer and over all primers was calculated for each single local variety and over all accessions. The observed number of alleles (n_o) and the effective number of alleles (n_e) per locus were calculated according to Kimura and Crow (1964). The polymorphism degree was calculated for each local variety and over all accessions using Shannon's information index (I) of phenotypic diversity (Lewontin 1972). Let p_i be the frequency of the i^{th} RAPD marker phenotype, the average diversity for a given accession can be written as follows: $I = -\Sigma p_i \ln p_i$.

Genetic diversity (H) and populations differentiation (D_{ST}) statistics of Nei (1973) were used to summarize the data of RAPD markers. Let p_i denote the frequency of the i^{th} marker allele at a given locus. The genetic diversity computed as $H = 1 - \Sigma p_i^2$ is equivalent to the expected heterozygosity. For a single locus, H ranges from 0 (monomorphic) to 1 (very highly discriminative with many alleles in equal frequencies). In measuring the extent of genetic differentiation, the total genetic diversity (H_T) over all loci and accessions considered together was first computed. From this, the proportion of diversity expressed between accessions (G_{ST}) was estimated as D_{ST}/H_T where D_{ST} is the among local variety differentiation computed as $H_T - H_S$ and thus $G_{ST} = 1 - H_S/H_T$. In the case of a single locus, H_S is the average over all accessions of the within local variety diversity at the locus. For estimates based on several

loci, H_S is the within local variety diversity over all loci and accessions of a given species.

Gene flow was estimated as follows: $Nm = 0.5\ (1 - G_{ST})/G_{ST}$. The result is independent of population size because the force of gene flow, which is measured by the fraction of migrants in a population (denoted as m), is counteracted by the force of genetic drift, which is proportional to the inverse of the population size (N). $Nm < 1$ indicates a local differentiation of populations, while $Nm > 1$ is evidence of a little differentiation among populations (McDermott and McDonald 1993).

All calculations and analyses were conducted using the software POPGENE version 1.21 (Yeh et al. 1997).

Dice (1945) genetic similarity (GS) estimates between individuals, based on the probability that a RAPD marker from one accession will also be present in another, was calculated in all possible pair-wise comparisons using the following formula: $GS_{ij} = 2M_{ij}/(2M_{ij} + M_i + M_j)$, where M_{ij} represents the number of shared amplification products scored between the pair of samples/fingerprints (i and j) considered, M_i is the number of products present in i but absent in j and M_j is the number of products present in j but absent in i. Thus, $GS_{ij} = 1$ indicates identity between i and j, whereas $GS_{ij} = 0$ indicates complete diversity. Mean genetic similarity (MGS) estimates of accession i to a set of accessions j was obtained by averaging single GS estimates according to the following formula: $MGS_{ij} = \Sigma_{ij}GS/n_j$ where n_j is the number of elements in set j.

The ordination analysis was performed according to the unweighted pair-group arithmetic average method (UPGMA) clustering algorithm (Sneath and Sokal 1973), and the dendrogram of single local varieties and centroids of all accessions were constructed from the symmetrical genetic similarity matrix. The principal coordinate analysis (PCOORDA) technique (Gower 1996) was applied to compute the first two components out of the qualitative data matrix. The triangular matrix of genetic similarity estimates was double-centered and then bi-dimentionally plotted according to the extracted Eigen-vectors (Rohlf 1972).

Genetic distance (GD) estimates among local varieties were calculated for RAPD markers using Nei (1978) unbiased genetic distance coefficient. This parameter is defined as: $GD_{ij} = -\ln \Sigma p_i\ p_j/(\Sigma p_i^2 \Sigma p_j^2)^{1/2}$, p_i and p_j being the frequencies of a given allele in populations i and j. For multiple loci these values are calculated by summing frequencies over alleles at all loci studied. $GD_{ij} = 1$ if no alleles are shared between populations i and j while a $GD_{ij} = 0$ indicates that the two populations have identical allele frequencies. Mean genetic distance (MGD) estimates of accession i to a set of accessions j was obtained by averaging single GD estimates according to the following formula: $MGD_{ij} = \Sigma_{ij}GD/n_j$ where n_j is the number of elements in set j.

All calculations and analyses were conducted using the appropriate routines of the software NTSYS version 1.80 (Rohlf 1993).

Results and discussion

Genetic diversity and differentiation statistics, along with gene flow estimates

Genetic variability was detected among plants of each local variety as well as among local varieties of emmer, as can be seen from the RAPD banding patterns of Figure 1. An average of 4.25 marker alleles per 10-mer primer were scored with a total number of polymorphisms of 17 in the emmer and 12 in the wheat materials (data not shown).

Descriptive statistics over all RAPD marker loci for single and grouped local varieties of emmer and for accessions of wheat are given in Table 2. Local variety ERSA≠7 showed the highest total number of marker loci per plant (12.6 ± 0.7) whereas the lowest one was found for ERSA≠8, which had 7.1 ± 0.5. In emmer, the observed number of marker alleles per locus (n_o) was 1.546 on average and 2 overall because 100% of marker loci showed to be polymorphic among all accessions, while the effective number of marker alleles per locus (n_e) was 1.735 (Table 2). In each local variety the level of polymorphism was highly variable and ranged from 23.5% of CAGN≠114 to 87.5% of ERSA≠4. Within-local variety monomorphic marker loci were scored over all accessions (on average, 45.0% in emmer and 55.9% in wheat).

The marker allele frequency showed great variability (Table 2). Marker alleles were highly variable in the emmer local varieties, with frequencies that individually ranged from 0.1 to 0.9 and that were on average higher than those of wheat (average p_i values over all accessions were 0.423 and 0.299, respectively). The lowest and the highest mean allele frequen-

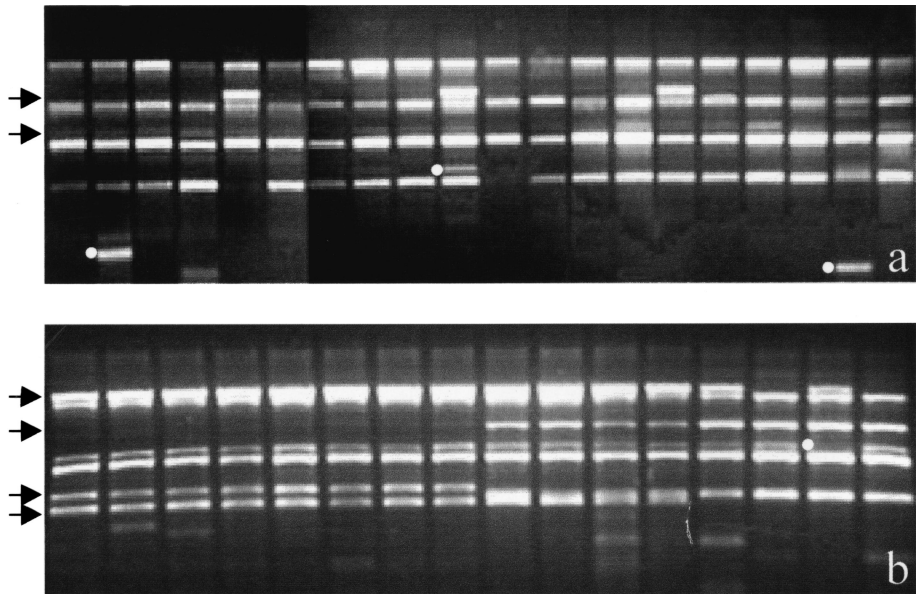

Figure 1. RAPD fingerprints of emmer accessions obtained using primers OPC-4 (a) and OPC-15 (b). Each plate includes two distinct accessions represented by 10 plants (a) and 8 plants (b) each. Arrows indicate between- and within-population polymorphisms. Bullets indicate single plant-specific polymorphisms.

cies were recorded by CAGN≠114 (0.167) and ERSA≠4 (0.679), respectively.

The Shannon's information index over all emmer accessions and RAPD marker loci was I = 0.595, varying from 0.115 of CAGN≠114 (very uniform) to 0.530 of ERSA≠4 (highly variable). Of the wheat accessions, «Belvedere» showed the highest genetic uniformity, the information index being as low as 0.143, while the other three accessions had comparable levels of diversity (Table 2).

Table 2. Descriptive statistics related to emmer and wheat including sample size, number of marker alleles per plant (No. m/p), % of polymorphic loci (% pl), observed (n_o) and expected (n_e) number of alleles, marker allele frequency (p_i), Shannon's information index (I) and Dice's genetic similarity (GS).

Accessions	Size	No. m/p	% pl	n_o	n_e	p_i	I	GS
ERSA≠1	10	11,2 ± 0,9	82.4	1.824	1.522	0.513	0.437	0.772
ERSA≠2	10	8,8 ± 1,1	58.8	1.588	1.466	0.621	0.357	0.698
ERSA≠3	10	8,5 ± 0,9	70.6	1.706	1.515	0.552	0.407	0.729
ERSA≠4	10	10,1 ± 1,0	87.5	1.824	1.695	0.679	0.530	0.752
ERSA≠5	10	9,8 ± 0,5	41.2	1.412	1.329	0.320	0.260	0.848
ERSA≠6	10	9,1 ± 1,2	52.9	1.529	1.366	0.373	0.297	0.846
ERSA≠7	10	12,6 ± 0,7	47.1	1.471	1.256	0.287	0.225	0.914
ERSA≠8	10	7,1 ± 0,5	64.7	1.647	1.411	0.513	0.350	0.686
LIVE≠105	10	11,1 ± 0,7	35.3	1.353	1.235	0.259	0.195	0.918
AMAT≠107	10	10,3 ± 0,6	41.2	1.412	1.311	0.374	0.248	0.913
CAGN≠114	10	10,7 ± 1,0	23.5	1.235	1.131	0.167	0.115	0.933
Overall emmer	110	9.8	55.0	2.000	1.735	0.423	0.595	0.699
sd		2.5	20.0	0.000	0.246	0.163	0.118	0.095
Belvedere	10	11,0 ± 1,0	29.4	1.294	1.158	0.087	0.143	0.931
Frassinese	10	11,0 ± 1,1	52.9	1.529	1.235	0.431	0.243	0.851
La Cappella	10	7,8 ± 0,7	41.2	1.412	1.266	0.261	0.224	0.905
Marzuolo	10	8,1 ± 0,4	52.9	1.529	1.247	0.419	0.236	0.849
Overall wheat	40	9.5	44.1	1.441	1.680	0.299	0.574	0.788
sd		1.8	11.3	0.113	0.220	0.161	0.132	0.041

Table 3. Genetic diversity and differentiation statistics along with gene flow estimates of emmer landraces based on RAPD marker loci.

Accessions	RAPD markers																	
	B7/1	B7/2	B7/3	B7/4	B17/1	B17/2	B17/3	C4/1	C4/2	C4/3	C4/4	C4/5	C15/1	C15/2	C15/3	C15/4	C15/5	H
ERSA≠1	0.097	0.097	0.495	0.433	0.000	0.498	0.000	0.273	0.465	0.433	0.433	0.000	0.349	0.097	0.498	0.444	0.414	0.296
ERSA≠2	0.000	0.000	0.444	0.000	0.349	0.494	0.000	0.495	0.494	0.465	0.000	0.000	0.000	0.097	0.494	0.495	0.414	0.250
ERSA≠3	0.433	0.494	0.208	0.488	0.494	0.097	0.000	0.494	0.000	0.000	0.000	0.000	0.414	0.208	0.444	0.494	0.498	0.281
ERSA≠4	0.433	0.273	0.414	0.495	0.495	0.000	0.433	0.000	0.433	0.495	0.495	0.433	0.000	0.483	0.483	0.483	0.488	0.373
ERSA≠5	0.000	0.000	0.000	0.000	0.349	0.000	0.000	0.000	0.433	0.000	0.495	0.000	0.000	0.494	0.414	0.433	0.465	0.181
ERSA≠6	0.000	0.475	0.500	0.000	0.000	0.000	0.108	0.444	0.000	0.000	0.000	0.000	0.137	0.444	0.444	0.457	0.444	0.203
ERSA≠7	0.108	0.108	0.444	0.000	0.414	0.000	0.000	0.433	0.000	0.433	0.000	0.000	0.097	0.494	0.000	0.000	0.000	0.149
ERSA≠8	0.433	0.000	0.000	0.000	0.232	0.000	0.433	0.097	0.273	0.000	0.000	0.494	0.414	0.495	0.433	0.208	0.494	0.236
LIVE≠105	0.500	0.000	0.433	0.349	0.452	0.000	0.000	0.000	0.000	0.000	0.000	0.433	0.097	0.000	0.000	0.000	0.000	0.133
AMAT≠107	0.189	0.000	0.494	0.433	0.380	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.498	0.452	0.000	0.470	0.171
CAGN≠114	0.000	0.000	0.495	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.097	0.273	0.000	0.433	0.076
H_S	0.199	0.132	0.357	0.200	0.288	0.099	0.088	0.203	0.191	0.166	0.129	0.124	0.137	0.310	0.358	0.274	0.375	0.214
H_T	0.376	0.321	0.487	0.436	0.483	0.456	0.359	0.429	0.485	0.453	0.480	0.177	0.164	0.473	0.484	0.419	0.486	0.410
D_{ST}	0.177	0.190	0.130	0.236	0.195	0.357	0.270	0.226	0.294	0.287	0.351	0.053	0.026	0.163	0.126	0.145	0.111	0.196
G_{ST}	0.471	0.590	0.267	0.542	0.404	0.783	0.753	0.526	0.607	0.634	0.731	0.302	0.161	0.344	0.260	0.346	0.229	0.479
Nm	0.562	0.347	1.376	0.423	0.738	0.139	0.164	0.450	0.324	0.289	0.184	1.157	2.615	0.951	1.423	0.945	1.687	0.544

Table 4. Dice's genetic similarity (GS) estimates (below diagonal) and Nei's genetic distance (GD) estimates (above diagonal) between pairwise accessions and mean estimates over all accessions.

	ERSA≠1	ERSA≠2	ERSA≠3	ERSA≠4	ERSA≠5	ERSA≠6	ERSA≠7	ERSA≠8	LIVE≠105	AMAT≠107	CAGN≠114	Mean estimates	Belvedere	Frassinese	La Cappella	Marzuolo	Mean estimates
ERSA≠1		0.555	0.686	0.364	0.449	0.390	0.270	0.702	0.654	0.358	0.294	0.472	0.485	0.514	0.388	0.422	0.452
ERSA≠2	0.741		0.582	0.211	0.525	0.337	0.454	0.622	0.367	0.583	0.456	0.417	0.523	0.487	0.695	0.633	0.585
ERSA≠3	0.587	0.479		0.197	0.375	0.311	0.442	0.530	0.393	0.514	0.425	0.393	0.588	0.594	0.578	0.540	0.575
ERSA≠4	0.660	0.624	0.649		0.155	0.304	0.178	0.467	0.277	0.242	0.253	0.265	0.352	0.394	0.387	0.513	0.412
ERSA≠5	0.670	0.743	0.545	0.713		0.309	0.177	0.494	0.492	0.239	0.235	0.345	0.391	0.509	0.441	0.592	0.483
ERSA≠6	0.707	0.719	0.547	0.609	0.676		0.103	0.649	0.360	0.199	0.203	0.316	0.291	0.304	0.469	0.334	0.349
ERSA≠7	0.791	0.792	0.600	0.729	0.788	0.834		0.486	0.276	0.090	0.099	0.257	0.223	0.259	0.409	0.445	0.334
ERSA≠8	0.533	0.548	0.438	0.592	0.527	0.506	0.600		0.337	0.472	0.698	0.546	0.390	0.324	0.419	0.448	0.395
LIVE≠105	0.649	0.594	0.603	0.661	0.649	0.717	0.794	0.583		0.216	0.437	0.381	0.412	0.285	0.546	0.515	0.439
AMAT≠107	0.767	0.778	0.512	0.676	0.742	0.793	0.866	0.530	0.785		0.127	0.304	0.364	0.357	0.439	0.504	0.416
CAGN≠114	0.821	0.790	0.616	0.732	0.758	0.800	0.876	0.522	0.702	0.857		0.322	0.434	0.477	0.460	0.562	0.483
Overall emmer	0.693	0.681	0.558	0.665	0.681	0.691	0.767	0.538	0.674	0.731	0.747		0.405	0.409	0.476	0.501	0.448
Belvedere	0.703	0.647	0.550	0.634	0.669	0.729	0.805	0.603	0.727	0.722	0.716	0.682		0.031	0.283	0.334	0.216
Frassinese	0.680	0.614	0.532	0.607	0.606	0.690	0.756	0.629	0.735	0.690	0.677	0.656	0.881		0.295	0.291	0.205
La Cappella	0.631	0.614	0.646	0.614	0.607	0.638	0.670	0.508	0.574	0.656	0.658	0.620	0.736	0.742		0.072	0.217
Marzuolo	0.571	0.528	0.516	0.581	0.473	0.657	0.627	0.541	0.593	0.565	0.584	0.567	0.674	0.724	0.797		0.232
Overall wheat	0.646	0.601	0.561	0.609	0.589	0.679	0.715	0.570	0.657	0.658	0.659	0.631	0.764	0.782	0.758	0.732	

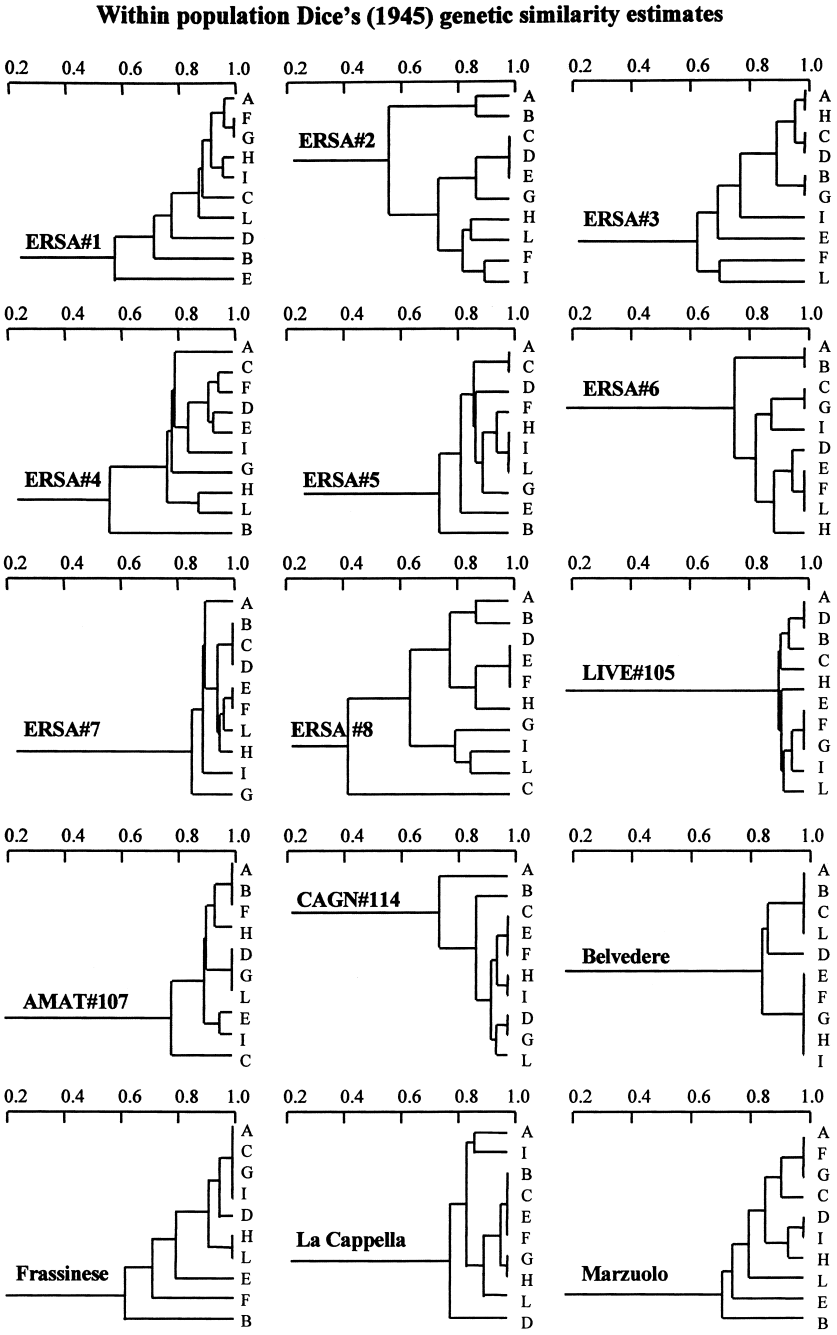

Figure 2. UPGMA dendrograms displaying the within-population Dice (1945) genetic similarity estimates.

Nei's genetic diversity estimates for all RAPD loci over single emmer local varieties are reported in Table 3. The total genetic diversity (H_T) for emmer was as high as 0.410. The average value of the within-population genetic diversity was $H_S = 0.214$, ranging from 0.076 of CAGN#114 to 0.373 of ERSA#4 (Table 3). This index varied between 0.093 and 0.154 in the wheat accessions, with a total genetic diversity of 0.390 (data not shown). The extent of differentiation between emmer accessions was $D_{ST} = 0.196$. Over all RAPD loci, the proportion of the among-local variety genetic diversity was as high as 48%

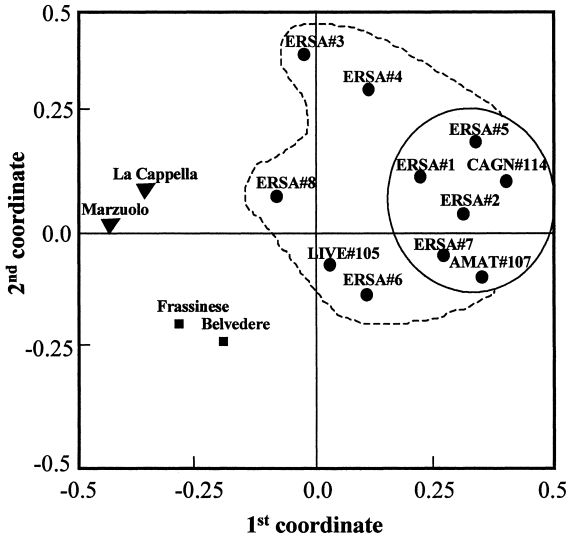

Figure 3. UPGMA centroids of emmer (bullets), common wheat (squares) and durum wheat (triangles) accessions defined according to the first two coordinates.

(G_{ST} = 0.479). Thus, about 52% of the total variation was within populations. The fixation index (G_{ST}) values calculated at each marker locus ranged from 0.161 (RAPD-C15/1) to 0.783 (RAPD-B17/2) and it was higher than 0.3 for 13 out of 17 loci (Table 3). The marked genetic differentiation among accessions over most of the loci was confirmed by the gene flow estimate that resulted as low as Nm = 0.544 (Table 3). On the whole, ERSA≠1 and ERSA≠4 were the local varieties that displayed the highest within-population genetic diversity; they showed no differentiation from the group of emmer accessions and an elevated gene flow (1.066 and 1.375, respectively). CAGN≠114, LIVE≠105 and ERSA≠7 were genetically the most uniform and differentiated local varieties.

Genetic similarities, genetic distances and ordination analyses

The Dice's genetic similarity (GS) within local varieties of emmer ranged from 0.686 of ERSA≠8 to 0.933 of CAGN≠114 and it was overall equal to 0.699 (Table 2).

GS and GD estimates between local varieties over all emmer and wheat accessions are reported in Table 4. The most similar emmer accessions were ERSA≠7, AMAT≠107 and CAGN≠114 (GS of about 0.870), while the most dissimilar accessions were ERSA≠3 and ERSA≠8 (GS = 0.438). Wheat local varieties displayed an average estimate of genetic similarity as high as 0.809. The mean genetic similarity (MGS) estimates varied from a minimum of 0.538 (ERSA≠8) to a maximum of 0.767 (ERSA≠7). The Nei's unbiased genetic distance (GD) varied between 0.090 (ERSA≠7 vs. AMAT≠107) and 0.702 (ERSA≠8 vs. ERSA≠1), with an average value of GD = 0.375. The emmer local variety classified as «Garfagnana» type (ERSA≠8) was the most differentiated from the rest of the accessions, its mean genetic distance being as high as MGD = 0.578. Local varieties of common and durum wheat showed pair-wise genetic distances of 0.031 and 0.072, respectively, while the mean genetic distance between them was 0.300 (Table 4).

Cluster analysis and dendrograms of single local varieties of emmer enabled one to visualize the relatively high level of the within population genetic dissimilarity of most accessions (Figure 2). Accessions ERSA≠7, LIVE≠105, AMAT≠107, and CAGN≠114 were the local varieties showing the highest levels of genetic similarity (GS varied between 0.913 and 0.933).

Principal components analysis allowed the definition of centroids from the mean genetic similarity matrix. All emmer local varieties were grouped separately from wheat accessions as can be seen from the scatter diagram plotted according to the first two components (Figure 3). It is worth mentioning that within emmer accessions, the ordination analysis based on the two principal axes allowed us to separate local varieties belonging to the «central Italy» group (ERSA≠1, ERSA≠2, ERSA≠5, ERSA≠7, AMAT≠107 and CAGN≠114) from those of the «southern Italy» group (ERSA≠3, ERSA≠4,

Table 5. Mean estimates of Dice's genetic similarity and Nei's genetic distance within and among emmer groups.

Emmer groups	Dice's genetic similarity		Nei's genetic distance	
	Central Italy	Southern Italy	Central Italy	Southern Italy
Central Italy	0.779	0.674	0.182	0.333
Southern Italy	0.674	0.631	0.333	0.351
Garfagnana	0.543	0.530	0.414	0.481

ERSA≠6, and LIVE≠105) and also from that of the «Garfagnana» type (ERSA≠8). The first three components with eigenvalues of 0.874, 0.476 and 0.403 were able to explain 67.4% of the total variation. In particular, the first component, which explains 33.6% of the total variation, was positively associated with emmer accessions from Central Italy and negatively associated with wheat accessions. The second component, which explains 18.3% of the total variation, was able to clearly distinguish durum wheat (La Cappella and Marzuolo) and common wheat (Frassinese and Belvedere) accessions. The most discriminant markers associated to the first coordinate were OPB-7/4 and OPC-4/1, whereas markers associated to the second coordinate were OPB-17/1.

Mean estimates of Dice's genetic similarity and Nei's genetic distance between group types of emmer «Central Italy», «Southern Italy» and «Garfagnana» are reported in Table 5. The mean genetic similarity between pair-wise comparisons was higher for central Italy (0.779) than for southern Italy (0.631) emmer local varieties. The same trend was observed in terms of genetic distances (0.182 for central Italy and 0.351 for southern Italy accessions). Thus, the central Italy group was much less variable and differentiated than the southern Italy group, even though the former includes a number of accessions higher than the latter (6 vs. 4). The Garfagnana-type local variety had genetic similarities and distances intermediate between central Italy and southern Italy group types (GS = 0.543 and 0.530, and GD = 0.414 and 0.481, respectively) (Table 5).

Conclusions

Local varieties of emmer proved to be formed by a variable number of lines genetically distinguishable among each other on the basis of RAPD fingerprints. The vast majority of individuals proved to be different multilocus genotypes. Each local variety of emmer scored from 60% (CAGN≠114) to 100% (ERSA≠4) of different haplotypes, while wheat accessions scored from 30% (Belvedere) to 70% (Marzuolo). Thus, the overall molecular marker data confirm the high variability that can be found within each emmer population, which strengthens the hypothesis that individuals belong to distinct local, more likely, folk varieties of a given landrace. If a landrace is a population managed by farmers, including seed selection, as stated by Zeven (1996), then a folk variety can be defined a single cultivar - morphotype - of that population (Stephen Brush, pers. comm.).

Landraces of emmer from central and southern Italy showed distinctive molecular traits. In particular, local varieties classified as «Central Italy» types were characterized by a common set of RAPD markers and proved to be distinguishable from either the «Souithern Italy» or the «Garfagnana» accessions. This result agrees with the characterization based on morpho-phenological and agronomic traits (D'Antuono and Pavoni 1993; Porfiri et al. 1998a, 1998c). Accessions ERSA≠1, ERSA≠2, ERSA≠5, ERSA≠7, AMAT≠105 and CAGN≠114 from central Italy showed a mean genetic diversity over all marker loci of 0.194, and a degree of genetic differentiation of 0.449. The same figures for accessions ERSA≠3, ERSA≠4, ERSA≠6 and LIVE≠105 from southern Italy were 0.247 and 0.407. The extents of between population genetic differentiation were as low as 0.175 and 0.122 for local variety groups from central and southern Italy, respectively. The within population diversity was 82% and 88% of the total genetic diversity. Local varieties belonging to the «Central Italy» group were much less variable and differentiated among each other than those of the «Southern Italy» group. The Garfagnana-type local variety had genetic similarities and distances intermediate between the two main group types. Accession ERSA≠8 from Garfagnana (Tuscany, central Italy) likely has a genetic background resembling those of emmer accessions from southern Italy. This finding is further supported by a number of morpho-phenological traits, mainly the winter growth habit, the medium size of the spike and the mixed floury-vitreous fracture of the kernels. Moreover, Garfagnana accession differs from the other emmer group types because of the presence of spikes either with or without awns. Such local variety is cultivated in a peculiar as well as isolated geographic area of Tuscany, in which farmers typically reproduce seed stocks over cropping seasons and do not exchange seed lots with foreigners.

Gene flow among plant populations can take place either by dispersal of pollen to a different population, successful fertilization of an ovule and establishment of the resulting seed within the site, or by dispersal of seed in a new site, germination and successful establishment of the deriving plants within the new population (Ennos 1994). The cleistogamy of emmer suggests that pollen dispersal and outcrossing is highly improbable, even though specific data on this topic are not available. In the case of local varieties of

emmer, gene flow among populations was probably caused by seed exchange among farmers.

Given the breeding system of emmer, genetic variation is expected to be greater among than within populations. Vice versa, our data indicate that more than half of the total genetic diversity (52%) is within populations. Similar results were also found for other selfers, such as landrace populations of barley (Papa et al. 1998) and wild populations of annual medics (Bonnin et al. 1996). In the case of emmer, selection carried out over the years by each farmer according to his own criteria produced a little genetic differentiation within the original populations. The marked genetic variation within population suggests that seed exchange among farmers might have occurred in each particular geographic area of cultivation. Moreover, more than 70% of the individuals studied were found to be different multilocus genotypes. These molecular results confirm the high variability that can be found within landrace populations, as previously reported by several authors for morpho-phenological traits (Castagna et al. 1996; Piergiovanni et al. 1996; D'Antuono and Minelli 1998; Cubadda and Marconi 1996; Laghetti et al. 1998; Porfiri et al. 1998b), and indicate that emmer local varieties are a mixture of a large number of distinct genotypes, despite the high level of selfing.

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References

Barcaccia G. 1994. Development, comparability and potential applications of RAPD markers in the genus *Medicago*. J. Genet. Breed. 48: 161–168.

Barcaccia G. and Rosellini D. 1996. A quick method for the isolation of plant DNA suitable for RAPD analysis. J. Genet. Breed. 50: 177–180.

Barcaccia G., Albertini E., Torricelli R., Russi L., Tomassini C., Negri V. et al. 1998. Caratterizzazione di antiche varietà locali di lenticchia e farro (with English summary) Atti del IV Congresso Nazionale Biodiversità September 8–11, 1998, Alghero, Italy, pp. 841–844.

Bonnin I., Huguet T., Gherardi M., Prosperi J.M. and Olivieri I. 1996. High level of polymorphism and spatial structure in a selfing plant species, *Medicago truncatula* (*Leguminosae*), shown using RAPD markers. Am. J. Bot. 83: 843–855.

Castagna R., Minoia C., Porfiri O. and Rocchetti G. 1996. Nitrogen level and seeding rate effects on the performance of hulled wheats (*Triticum monococcum* L., *T. dicoccum* Schubler and *T. spelta* L.) evaluated in contrasting agronomic environments. J. Agron. and Crop Sci. 176: 173–181.

Cubadda R. and Marconi E. 1996. Technological and nutritional aspects in emmer and spelt. In: Padulosi S., Hammer K. and Heller J. (eds), Hulled wheats. Promoting the conservation and use of underutilized and neglected crops. Proc. of 1st Intl. Workshop on Hulled Wheats, July, 21–22, 1995. Castelvecchio Pascoli, Lucca, Italy, pp. 203–211.

D'Antuono L.F. and Pavoni A. 1993. Phenology and grain growth of *Triticum dicoccum* and *T. monococcum* from Italy. In: Damania A.B. (ed.), Biodiversity and Wheat Improvement. Wiley-Sayce, UK, pp. 273–286.

D'Antuono L.F. and Minelli M. 1998. Yield and yield components analysis of emmer wheat (*Triticum dicoccum* Schubler) landraces from Italy. In: Jaradat A.A. (ed.), Proc. of the 3rd Intl. Triticeae Symposium, May, 4–8, 1997, Aleppo, Syria. Science Publisher, Inc., USA, pp. 393–404.

Dice L.R. 1945. Measures of the amount of ecological association between species. Ecology 26: 297–302.

Ennos R.A. 1994. Estimating the relative rates of pollen and seed migration among plant populations. Heredity 72: 250–259.

Gower J.C. 1996. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53: 325–338.

Kimura M. and Crow J.F. 1964. The number of alleles that can be maintained in a finite population. Genetics 49: 725–738.

Laghetti G., Piergiovanni A.R., Perrino P. and Blanco A. 1998. Agronomic and nutritional characteristics in emmer and spelt. In: Slinkard A.E. (ed.), Proc. of 9th Int. Wheat Genetic Symposiun, August, 2–7, 1998. University Extension Press, Saskatoon, Saskatchewan, Canada 2:, pp. 270–272.

Lewontin R.C. 1972. The genetic basis of evolutionary change. Columbia Univ. Press.

McDermott J.M. and McDonald B.A. 1993. Gene flow in plant pathosystems. Ann. Rev. Phytopathol. 31: 353–373.

Nei M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA 70: 3321–3323.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583–590.

Papa R., Attene G., Barcaccia G., Ohgata A. and Konishi T. 1998. Genetic diversity in barley (*Hordeum vulgare* L.) landrace populations from Sardinia, Italy, as revealed by RAPDs, isozymes and morpho-phenological traits. Plant Breed. 117: 523–530.

Piergiovanni A.R., Laghetti G. and Perrino P. 1996. The characteristics of meal from hulled wheats (*Triticum dicoccum* Schrank and *T. spelta* L.): evaluation of selected accessions. Cereal Chemistry 73: 732–735.

Porfiri O., Papa R. and Veronesi F. 1998a. Il farro nel rilancio delle aree marginali umbro-marchigiane (with English summary). In: Papa C. (ed.), Il farro. Saperi, Usi e Conservazione Delle Varietà Locali. Quaderni del CEDRAV 1, pp. 58–67.

Porfiri O., D'Antuono L.F. and Perrino P. 1998b. Stato della ricerca e della sperimentazione sui frumenti vestiti in Italia, con particolare riferimento al farro medio (*Triticum dicoccum* Schubler). (with English summary). In: Troccoli A., De Vita P. and Di Fonzo N. (eds), Prospettive di una moderna cerealicoltura alle

Porte del terzo millennio, V. Int. Workshop on durum wheat, April 30–May 2, 1998, Foggia, Italy, pp. 327–351.

Porfiri O., D'Antuono L.F., Codianni P., Mazza L. and Castagna R. 1998c. Genetic variability of a hulled wheats collection for agronomic and quality characteristics. In: Jaradat A.A. (ed.), Proc. of the 3rd Int. Triticeae Symposium, May, 4–8, 1997, Aleppo, Syria. Science Publisher, Inc., USA, pp. 387–392.

Rohlf F.J. 1972. An empirical comparison of three ordination techniques in numerical taxonomy. Systematic Zool. 21: 271–280.

Rohlf F.J. 1993. NTSYS-pc: Numerical taxonomy and multivariate analysis system. Version 1.8. State University of New York, Stony Brook, NY.

Sambrook J., Fritsch E.F. and Maniatis T. 1989. Molecular cloning, a laboratory manual. 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sneath P.H.A and Sokal R.R. 1973. Numerical taxonomy. W.H. Freeman and Company, San Francisco, CA.

Szabo A.T. and Hammer K. 1996. Notes on the taxonomy of farro: *Triticum monococcum*, *T. dicoccum* and *T. spelta*. In: Padulosi S., Hammer K. and Heller J. (eds), Hulled wheats. Promoting the conservation and use of underutilized and neglected crops. Proc. of 1st Int. Workshop on Hulled Wheats, July, 21–22, 1995, Castelvecchio Pascoli, Lucca, Italy, pp. 2–40.

U.P.O.V 1974. Guidelines for conduction of tests for distinctiveness, homogeneity and stability. Wheat (*Triticum aestivum* L., *Triticum durum* Desf.) TG/3/1, Geneve.

Yeh F.C., Yang R.C. and Boyle T. 1997. POPGENE Version 1.21. CIFOR and University of Alberta, Canada.

Williams J.G.K., Kubelik A.R., Livak K.J., Rafalski J.A. and Tingey S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 22: 6531–6535.

Zeven A.C. 1996. Results of activities to maintain landraces and other material in some European countries *in situ* before 1945 and what we may learn from them. Genet. Resour. Crop Evol. 43: 337–341.