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Supplementary material

Morphological and molecular characterization of ancient pomegranate (*Punica granatum* L.) accessions in Northern Italy

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All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

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Abstract: The Italian research on *P. granatum* L. is still limited, although the study of local germplasm is extremely important in order to preserve the existing biodiversity and to identify potential useful characters for a renewed industry. The study aimed at characterizing for the first time ancient pomegranates, grown in Emilia Romagna (Italy), through 38 quantitative morphometric descriptors related to leaf, flower, fruit and seed, 42 RAPD and 12 SSR markers. Morphological analyses showed large variation of traits among accessions and the descriptors related to fruit and seed had the highest power of discrimination. The considerable variation found was consistent with ANOVA and PCA results. Among all RAPDs tested, 7 were selected for their polymorphism; whereas among selected SSRs, 8 presented differences in the genetic profiles allowing a good discrimination of the local pomegranate accessions. The genetic relationships among pomegranates were studied by UPGMA cluster analysis and the accessions were clearly regrouped in four different genotypes. The study has highlighted significant differences and interesting pomological characteristics in the local pomegranates, which confirmed the good potential of this germplasm for the pomegranate industry.

Table S1 - List of decamer random oligonucleotide primers valued in this study

Primer	Sequence	Primer	Sequence
OPA01	5′-d[CAGGCCCTTC]-3′	OPP10	5′-d[TCCCGCCTAC]-3′
OPA02	5′-d[TGCCGAGCTG]-3′	OPX01	5′-d[CTGGGCACGA]-3′
OPA03	5′-d[AGTCAGCCAC]-3′	OPX03	5′-d[TGGCGCAGTC]-3′
OPA04	5′-d[AATCGGGCTG]-3′	OPX09	5′-d[GGTCTGGTTG]-3′
OPA05	5′-d[AGGGGTCTTC]-3′	OPC16	5′-d[CACACTCCAG]-3′
OPA07	5′-d[GAAACGGGTG]-3′	544	5′-d[TAGAGACTCC]-3′
OPA09	5′-d[GGGTAACGCC]-3′	AH01	5′-d[TCGGCAACCA]-3′
OPA10	5′-d[GTGATCGCAG]-3′	AH02	5′-d[CACTTCCGCT]-3′
OPA11	5′-d[CAATCGCCGT]-3′	AH09	5′-d[AGAACCGAGG]-3′
OPA12	5′-d[TCGGCGATAG]-3′	AH11	5′-d[TCCGCTGAGA]-3′
OPA16	5′-d[AGCCAGCGAA]-3′	AH12	5′-d[TCCAACGGCT]-3′
OPA19	5′-d[CAAACGTCGG]-3′	AH16	5′-d[CAAGGTGGGT]-3′
OPA20	5′-d[GTTGCGATCC]-3′	AH17	5′-d[CAGTGGGGAG]-3′
OPB08	5′-d[GTCCACACGG]-3′	AH18	5′-d[GGGCTAGTCA]-3′
OPB10	5′-d[CTGCTGGGAC]-3′	AH19	5′-d[GGCAGTTCTC]-3′
OPB12	5′-d[CCTTGACGCA]-3′	AI08	5′-d[AAGCCCCCCA]-3′
OPB13	5′-d[TTCCCCCGCT]-3′	AI05	5′-d[GTCGTAGCGG]-3′
OPB16	5′-d[TTTGCCCGGA]-3′	AI11	5′-d[ACGGCGATGA]-3′
OPB19	5′-d[ACCCCCGAAG]-3′	AI12	5′-d[GACGCGAACC]-3′
OBB20	5′-d[GGACCCTTAC]-3′	AI13	5′-d[ACGCTGCGAC]-3′
OPK16	5′-d[GAGCGTCGAA]-3′	AI14	5′-d[TGGTGCACTC]-3′

Table S2 - Mean values, standard deviation and ANOVA analysis for leaf characteristics

ID	LL[(z)]	LD	LFW	LS
ME1	6.92 ±0.90 A	2.15 ±0.37 A	0.12±0.03 B	3.28±0.58 AB
ME2	6.20 ±1.21 AB	1.68 ±0.42 BC	0.16±0.06 A	3.91±1.25 A
ME3	4.64 ±0.49 CD	1.35±0.27 DE	0.09±0.02 CD	3.59±0.91 AB
ME4	5.70 ±1.23 BC	1.48 ±0.16 CDE	0.11±0.03 BC	3.90±1.03 A
ME5	4.45 ±0.90 D	1.26 ±0.27 E	0.07±0.02 D	3.74±1.30 AB
ME6	4.98 ±0.63 CD	1.72 ±0.27 BC	0.10±0.03 BC	2.97±0.62 B
ME7	5.55±0.81 BC	1.76 ±0.24 B	0.09±0.02 CD	3.22±0.67 AB
ME8	5.10 ±1.17 CD	1.54 ±0.34 BCD	0.07±0.02 D	3.38±0.59 AB

The same letter show no statistically significant differences (P<0.05).
[(z)] For explanation of character symbols, see table 2.

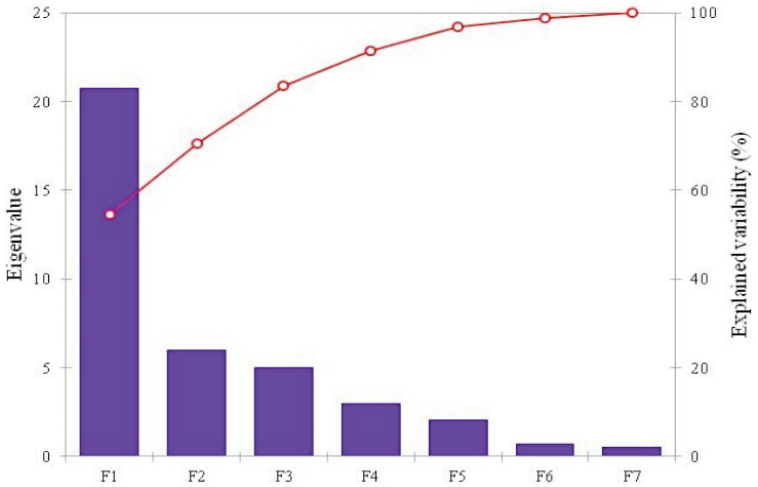

Fig. S1- Screen plot obtained from the PCA (F1-F7 denote the seven principal components) for total traits studied.

Table S3 - Mean values, standard deviation and ANOVA analysis for long-styled and short-styled flower characteristics

ID	FDL[z]	FLL	PNL	PLL	FDS	FLS	PNS	PLS
ME1	1.52±0.15 AB	4.80±0.44 A	7.5±0.55 A	1.67±0.39 A	1.39±0.14 BC	3.57±0.45 BC	7.40±0.52 A	0.60±0.23 A
ME2	1.75±0.21 AB	5.95±0.07 A	6.0±0 AB	1.50±0.28 A	1.47±0.05 ABC	3.60±0.41 BC	6.25±0.50 BC	0.37±0.22 AB
ME3	1.40±0.35 AB	5.47±1.28 A	5.67±0.58 B	2.03±0.06 A	1.55±0.13 AB	4.54±0.55 A	6.89±0.78 ABC	0.34±0.12 AB
ME4	1.81±0.20 A	5.44±0.78 A	7.43±0.98 A	1.50±0 A	1.50±0.20 ABC	4.10±0.43 AB	6.33±0.58 ABC	0.40±0.51 AB
ME5	1.70±0 AB	5.60±0.28 A	6.0±0 AB	1.86±0.33 A	1.53±0.25 ABC	3.97±0.29 AB	6.22±0.60 BC	0.31±0.16 B
ME6	1.45±0.07 AB	4.95±0.07 A	7.0±0 AB	1.95±0.07 A	1.77±0.12 A	4.18±0.44 AB	6.91±.070 AB	0.38±0.15 AB
ME7	1.22±0.19 B	2.90±0.31 B	6.6±0.55 AB	1.97±0.05 A	1.20±0.07 C	2.84±0.39 C	6.0±0.63 BC	0.55±0.06 AB
ME8	1.27±0.25 B	2.92±0.20 B	6.0±0 B	1.50±0.61 A	1.39±0.34 BC	3.10±0.59 C	6.0±0 C	0.50±0.14 AB

The same letter show no statistically significant differences (P<0.05).
[z] For explanation of character symbols, see table 2.

Table S4 - Mean values, standard deviation and ANOVA analysis for seed and tegmen characteristics

ID	STW [z]	SL	SD	SW	TL	TD	TW	WPI	% A	SL/SD	TL/TD	AW	AW/TW
ME1	168.438 ±31.68 A	0.970 ±0.09 B	0.721 ±0.09 B	0.381 ±0.06 A	0.618 ±0.06 D	0.294 ±0.05 CD	0.028 ±0 A	0.076 ±0.01 B	92.4 ±0.01 A	1.35 ±0.23 B	2.10 ±0.58 AB	0.35 ±0.05 A	12.39 ±1.75 A
ME2	159.112 ±43.76 AB	1.068 ±0.08 A	0.804 ±0.07 A	0.295 ±0.05 C	0.720 ±0.06 B	0.349 ±0.05 B	0.025 ±0 B	0.086 ±0.01 AB	91.2 ±0.01 AB	1.32 ±0.15 B	2.06 ±0.35 AB	0.27 ±0.04 AB	10.79 ±1.56 AB
ME3	168.244 ±12.64 A	0.944 ±0.09	0.698 ±0.11 BC	0.283 ±0.05 C	0.656 ±0.05 C	0.308 ±0.05 C	0.027 ±0 B	0.097 ±0.03 AB	90.3 ±0.03 AB	1.33 ±0.23 B	2.09 ±0.52 AB	0.26 ±0.01 AB	9.41 ±2.52 AB
ME4	202.556 ±85.82 A	1.08 ±0.09 A	0.724 ±0.13 B	0.342 ±0.08 B	0.690 ±0.08 B	0.293 ±0.06 CD	0.026 ±0 B	0.071 ±0.01 B	92.6 ±0.01 A	1.54 ±0.27 A	2.41 ±0.55 A	0.32 ±0.06 AB	12.39 ±2.85 A
ME5	163.885 ±41.95 A	0.982 ±0.12 B	0.668 ±0.12 C	0.290 ±0.05 C	0.656 ±0.06 C	0.274 ±0.05 DE	0.022 ±0 C	0.076 ±0 AB	92.4 ±0 A	1.47 ±0.61 AB	2.40 ±0.59 AB	0.27 ±0.01 AB	12.14 ±0.6 AB
ME6	156.037 ±30.46 AB	1.045 ±0.06 A	0.788 ±0.07 A	0.266 ±0.04 C	0.769 ±0.05 A	0.416 ±0.04 A	0.029 ±0.04 A	0.118 ±0.01 A	88.9±0.02 AB	1.31 ±0.15 B	1.88 ±0.25 B	0.24 ±0BC	8.15 ±0.4 AB
ME7	58.076 ±14.43 B	0.804 ±0.07 C	0.584 ±0.08 D	0.168 ±0.01 D	0.575 ±0.06 E	0.277 ±0.04 CDE	0.018 ±0 D	0.118 ±0.01 A	88.2 ±0 B	1.39 ±0.24 AB	2.13 ±0.46 AB	0.14 ±0 C	7.64± 0.03 B
ME8	58.998 ±16.08 B	0.786 ±0.07 C	0.547 ±0.08 D	0.144 ±0.01 D	0.544 ±0.05 E	0.253 ±0.04 E	0.016 ±0 D	0.121 ±0.01 A	88.8 ±0 AB	1.44 ±0.28 AB	2.16 ±0.33 AB	0.12 ±0 C	7.40 ±0.02 B

The same letter show no statistically significant differences (P<0.05).
[z] For explanation of character symbols, see table 2.

Table S5 - Eigenvalues, percentage of variance and percentage cumulative associated with the principal components, for total traits studied in the local pomegranates

Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7
FW	0.986	0.044	-0.010	-0.135	0.012	-0.079	0.031
FD	0.983	-0.058	-0.081	-0.129	0.012	0.067	0.041
CD	0.843	0.219	0.070	-0.246	-0.327	0.225	-0.135
FL1	0.966	0.161	-0.102	0.139	0.027	0.026	0.095
FL2	0.984	0.042	-0.015	0.064	-0.083	0.134	-0.037
CL	0.771	-0.231	0.542	-0.106	-0.080	0.134	-0.149
FT	0.888	-0.044	0.330	0.193	-0.243	-0.058	-0.002
SCW	0.974	0.107	0.038	-0.191	-0.045	-0.029	0.001
NC	0.923	-0.117	0.004	-0.313	-0.037	-0.186	0.024
STW	0.974	-0.001	-0.123	-0.030	0.116	-0.129	0.072
SL	0.887	-0.099	-0.170	0.316	0.273	0.012	0.015
SD	0.803	-0.403	-0.172	0.389	0.078	0.075	0.013
SW	0.956	-0.101	0.248	-0.095	0.029	-0.049	0.054
TL	0.660	-0.344	-0.465	0.352	0.307	0.101	0.031
TD	0.334	-0.716	-0.420	0.375	0.224	0.091	-0.019
TW	0.825	-0.518	-0.119	-0.019	0.053	-0.138	0.122
WPI	-0.855	-0.411	-0.301	0.074	0.016	-0.055	-0.038
A%	0.802	0.558	0.015	0.010	0.116	0.132	0.121
SL/SD	-0.015	0.802	0.161	-0.185	0.507	-0.195	0.026
TL/TD	0.196	0.903	0.164	-0.248	0.227	-0.001	0.080
AW	0.965	-0.071	0.220	-0.096	0.035	-0.052	0.049
AW/TW	0.862	0.340	0.342	-0.109	0.056	0.092	-0.029
FSI	-0.322	0.641	-0.006	0.691	-0.005	-0.076	0.049
CSI	-0.254	-0.128	0.360	-0.212	0.774	0.358	-0.129
SC%	0.935	0.115	0.074	-0.243	-0.161	0.058	-0.133
S%	-0.935	-0.116	-0.078	0.242	0.164	-0.055	0.131
FDL	0.838	0.375	0.012	0.301	0.228	0.084	-0.085
FLL	0.934	0.064	-0.299	0.144	-0.080	0.081	-0.010
PNL	0.277	-0.339	0.590	-0.078	0.632	-0.164	0.168
PLL	-0.127	-0.390	-0.475	-0.546	-0.107	0.354	0.415
FDS	0.500	-0.338	-0.665	-0.016	0.286	-0.150	-0.300
FLS	0.727	-0.102	-0.588	-0.210	0.044	-0.256	0.064
PNS	0.549	-0.688	0.160	-0.356	-0.163	-0.213	-0.027
PLS	-0.430	-0.290	0.840	-0.114	-0.026	-0.095	0.049
LL	0.246	-0.262	0.856	0.355	-0.088	-0.042	0.047
LW	-0.012	-0.653	0.745	0.117	-0.005	0.059	-0.020
LFW	0.506	-0.260	0.301	0.733	-0.177	0.058	0.119
LS	0.490	0.773	-0.051	0.338	-0.192	-0.055	0.076
Eigenvalue	20.75	6.01	4.98	2.98	2.06	0.73	0.49
Variance %	54.60	15.82	13.11	7.84	5.42	1.92	1.28
Cumulative %	54.60	70.43	83.53	91.38	96.80	98.72	100

Table S6 - Genetic profiles of eight pomegranate accessions. Columns list the allele sizes for the 12 investigated loci (PGKVR-, POMA-, PG, POM, PGAER)

Accessions	SSRs loci4											
	PGKVR027	PGKVR064	PGKVR065	PGKVR114	PGKVR127	PGKVR165	POMAGC11	PG4	PG6	POM021	POM045	PGAER154
ME1	242/242	239/241	204/204	258/258	246/246	307/319	185/185	198/244	193/193	209/211	157/157	264/300
ME2	242/242	239/241	202/204	258/258	246/246	307/319	183/185	198/244	193/195	203/205	155/157	262/300
ME3	242/242	239/241	202/204	258/258	246/246	307/319	183/185	198/244	193/195	203/205	155/157	262/300
ME4	242/242	239/241	202/204	258/258	246/246	307/319	183/185	198/244	193/195	203/205	155/157	262/300
ME5	242/242	239/241	202/204	258/258	246/246	307/319	183/185	198/244	193/195	203/205	155/157	262/300
ME6	242/242	239/241	204/204	258/258	246/246	307/319	185/185	198/244	191/193	205/207	157/163	262/282
ME7	236/236	241/241	204/204	258/258	246/246	307/319	185/185	198/244	197/199	205/207	163/163	262/300
ME8	236/236	241/241	204/204	258/258	246/246	307/319	185/185	198/244	197/199	205/207	163/163	262/300

Accessions are grouped by genotype, different genotypes are separated by horizontal lines.