Evaluation of Genetic Diversity in the Pistacia Germplasm Collection of the National Fruit Germplasm Centre(NFGC) Using EST-SSRs

N. Santillo [(1)], S. Micali [(1)], E. Vendramin [(1)]

Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - Olive, Citrus and Fruit Crops, [(1)],Italy

BACKGROUND

Collecting, maintaining, characterizing and exchanging of plant genetic resources for food and agriculture is crucial to prevent the agrobiodiversity loss. Germplasm collections are a pivotal source of useful genes to answer to challenging issues such as climate changes and emerging pests and diseases. The CREA- Centro di Ricerca Olivicoltura, Frutticolturae Agrumicoltura in Rome hosts the National Fruit Germplasm Centre (NFGC) that maintains, in the frame of the International Treaty of Plant Genetic Resources for Food and Agriculture, the largest national fruit germplasm collection composed of more than 5.000 accessions (each one duplicated) belonging to 40 different fruit species and their wild relatives. The genus *Pistacia* of the Anacardiaceae family consists of at least eleven species with dioecious plants (Zohary,1952). Among the *Pistacia* genus, only *P. vera* L. has edible nuts and commercial importance, while the other species are mainly used as rootstocks (*P. integerrima*, *P mutica*, *P. terebinthus and P. atlantica*) or as pollinators (*P. terebinthus*). Dioecism represents an inconvenience to pistachio breeding and the long juvenile period (5-8 years) hampers sex determination until flowering. In this outline a molecular marker linked to sex could facilitate breeding allowing early seedling selection with the saving of time and economic resources. Some cases of monoecism have been reported for *P. vera*, *P. atlantica* and *P. terebinthus*. Germplasm collections are an important tool for plant breeding, the chance to observe different genotypes in the same environment could help to identify the best parents for breeding programs. The NFGC Pistacia collection was established in the 2006 and it is composed of 120 accessions, belonging to 24 *P. vera* cultivars, 13 accessions to *P. integerrima* and four to *P. terebinthus*.

OBJECTIVES

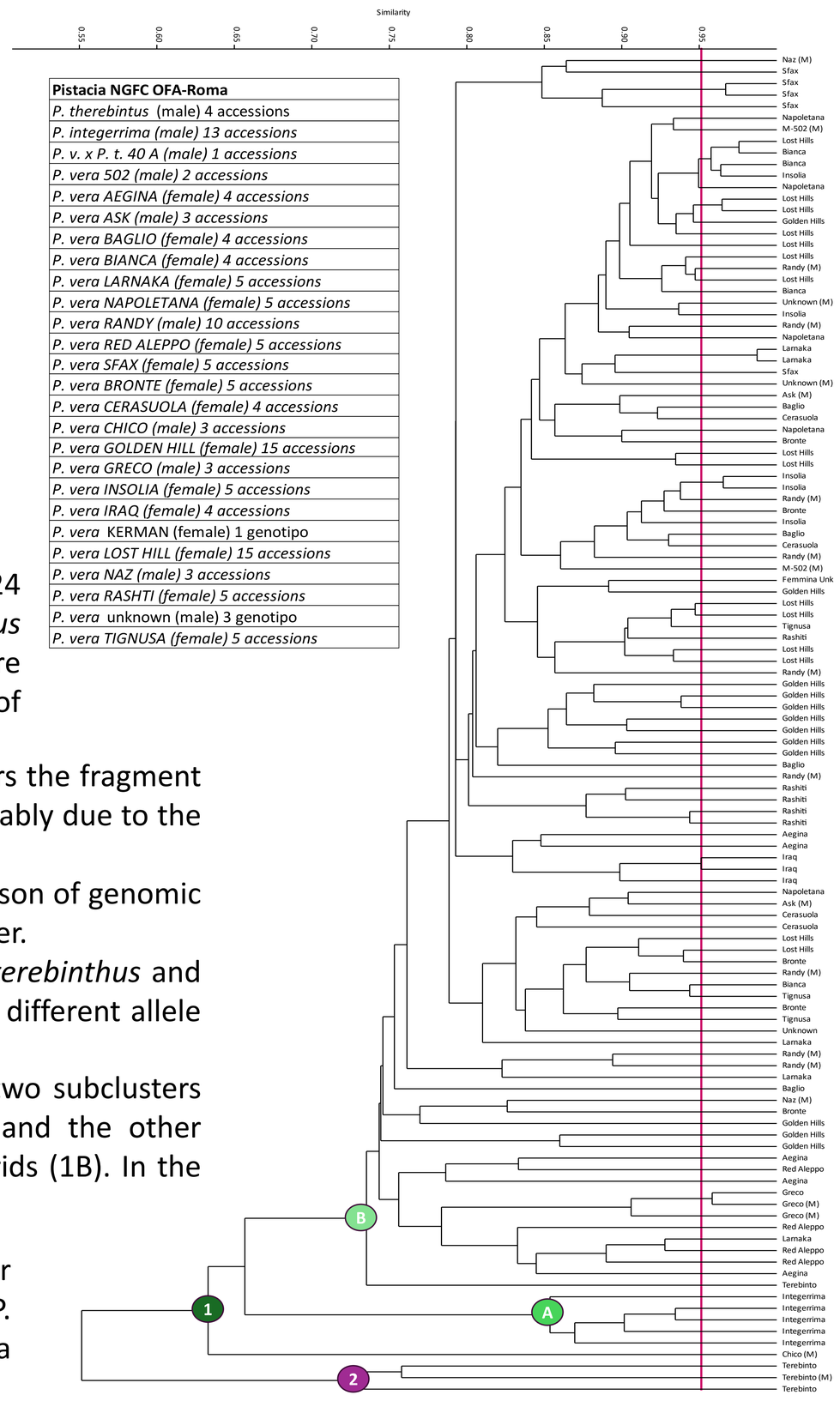
The aim of the work is to evaluate the genetic diversity within the collection and to identify and resolve synonymies and homonymies by using 30 EST-SSRs loci previously developed in *P. vera* L. from male and female inflorescences.

MATERIALS AND METHODS

Genomic DNA was extracted from young apical shoots, after lyophilization, using the GENEzol™ DNA Reagent Plant (Geneaid) with modified protocol. Genomic DNAs were quantified both on agarose gel and by using the spectrophotometer to check quality and integrity. The DNA samples were diluted to a final concentration of 10 ng/ul. Thirty EST-SSR loci, belonging to the EPV series, were amplified using annealing temperature, touch-down PCR cycles and protocol as reported in Anderson et al., 2010. The EPV EST-SSR loci were identified starting from two different libraries of expressed sequence tags deriving from male and female flowers of *P. vera*, respectively. PCR products were visualized on capillary electrophoresis (CEQ8800 – Beckman) if comprised between 100-300 bp and on a high-resolution agarose gel (MetaPhor™ - Lonza).

Cluster analysis was performed with Past 5.0.2 (Hammer, Ø. Et al., 2001) and a similarity matrix was constructed, after filtering for samples with missing data > 20%, according to Dice index. Dendrograms were obtained by the UPGMA method.

Pistacia NGFC OFA-Roma
P. therebintus (male) 4 accessions
P. integerrima (male) 13 accessions
P. v. x P. t. 40 A (male) 1 accessions
P. vera 502 (male) 2 accessions
P. vera AEGINA (female) 4 accessions
P. vera ASK (male) 3 accessions
P. vera BAGLIO (female) 4 accessions
P. vera BIANCA (female) 4 accessions
P. vera LARNAKA (female) 5 accessions
P. vera NAPOLETANA (female) 5 accessions
P. vera RANDY (male) 10 accessions
P. vera RED ALEPPO (female) 5 accessions
P. vera SFAX (female) 5 accessions
P. vera BRONTE (female) 5 accessions
P. vera CERASUOLA (female) 4 accessions
P. vera CHICO (male) 3 accessions
P. vera GOLDEN HILL (female) 15 accessions
P. vera GRECO (male) 3 accessions
P. vera INSOLIA (female) 5 accessions
P. vera IRAQ (female) 4 accessions
P. vera KERMAN (female) 1 genotipo
P. vera LOST HILL (female) 15 accessions
P. vera NAZ (male) 3 accessions
P. vera RASHTI (female) 5 accessions
P. vera unknown (male) 3 genotipo
P. vera TIGNUSA (female) 5 accessions



RESULTS

Locus Code	Motif	Allele range	# Alleles
EPVF007	$(gtga)_3$	215-232	3
EPVF010	$(ctgt)_3$	208-216	3
EPVF013	$(ct)_{10}$	500-515	4
EPVF018	$(gct)_5$	510-520	2
EPVF021	$(taatg)_3$	219-228	3
EPVF023	$(aaag)_3$	191-245	8
EPVF030	$(tgg)_4$	650-675	2
EPVF031	$(ttatg)_7$	220-245	5
EPVF032	$(ct)_5$	480-500	6
EPVM002	$(tgc)_5$	750-766	3
EPVM016	$(aag)_4$	500-510	5
EPVM017	$(ag)_8$	245-249	2
EPVM022	$(tc)_6$	238-250	5
EPVM024	$(agacc)_3$	227-248	5
EPVM029	$(cct)_4$	115-160	10
EPVM033	$(ac)_5$	302-304	2
EPVM035	$(aga)_4$	140-143	2
EPVM040	$(ggaga)_3$	62-116	4
EPVM041	$(tgtga)_3$	307-330	4
EPVM043	$(ttat)_3$	770-780	2
EPVM049	$(att)_4$	249-291	6
EPVM050	$(aga)_4$	780-795	2
EPVM051	$(aga)_4$	110-149	4
EPVM054	$(gat\text{-}cac)_5$	770-790	2
EPVM056	$(tgg\text{-}ggt)_4$	480-500	7
EPVM058	$(tc)_6$	245-275	4
EPVM059	$(ttatg)_4$	220-245	5
EPVM063	$(tg)_5$	157-190	7

The NFGC Pistacia collection composed of 120 accessions, belonging to 24 P. vera cultivars, 13 accessions to *P. integerrima* and four to *P. terebinthus* was characterized using 30 EST-SSRs. Twenty-eight out of them were polymorphic (Tab1) and a total of 107 fragments were obtained, 100 of these were polymorphic.

The fragments size ranged from 110 to 790 bp, and for the half of markers the fragment size observed was considerably higher than the expected one, this is probably due to the presence of introns.

As expected for EST-SSRs the level of polymorphism was lower in comparison of genomic microsatellites, but the rate of transferability across related species is higher.

Characterising species alleles were identified, in particular eleven for *P. terebinthus* and eight for *P. integerrima*, no specific alleles were identified for *P. vera* but different allele combinations.

The cluster analysis revealed two main groups (1, 2). Within cluster 1 two subclusters were identified: one grouping all the *P. Integerrima* genotypes (1A) and the other gathering all the P. vera genotypes and the *P. vera x P. terebinthus* hybrids (1B). In the second cluster are located all the *P. therebintus* accessions (2).

All the P. vera genotypes and a *P. vera* x *P. terebinthus* hybrid were brought together in subcluster 1A independently from their gender, excepted for Chico that is located near *P. therebintus* and P. integerrima. This result is unexpected due to the morphological aspect of the tree that is clearly a Pistacia vera, probably some mislabelling error was occurred during the field sampling.

Genotypes with identical names do not show identical genetic profiles, also if they are closely located in the phylogenetic tree, this i probably due to the origin of the collection. For the Bianca or Napoletana cultivar, the green kernel one, is well known that this is a population cultivar and for this reason genetic diversity between genotypes with the same name is expected.

The NFGC Pistacia collection was established at the end of 90th century, collecting sticks and seed from different mediterranean and north-east region collections and from different cultivated fields and natural sites in Italy. Due to the collection's nature some differences between samples that share the same name is expected, as for the higher genetic diversity observed between the traditional Italian P. vera varieties (Bianca, Napoletana, Insolia, Bronte e Cerasuola).

SUMMARY AND PERSPECTS

The genetic characterization allowed to clearly identify different species belonging to Pistacia genus and to identify ambiguous situations that require further study in order to be sure of preserving what is really necessary and rationalizing the collection.

Progetto "Azioni di VALOrizzazione e REcupero per le filiere Italiane di Nocciolo, CAstagno, Mandorlo,Pistacchio e carrubO - VALO.RE I.N. CA.M.P.O" - Finanziato dal MASAF D.D. N. 667521 del 30.12.2022

MINISTERO DELL'AGRICOLTURA DELLA SOVRANITÀ ALIMENTARE E DELLE FORESTE