New conventions for DNA fingerprinting: methodological workflows and future perspectives

geneticaagraria.it

NUNZIATA A.[1], D'AGOSTINO N.[2], DE MASI L.[3], CICE D.[1], FERRARA E.[4], MAGRI A.[4] AND PETRICCIONE M.[1]

1) Centro di Ricerche Olivicoltura, Frutticoltura e Agrumicoltura, CREA, Caserta, Italy
2) Dipartimento di Agraria, Università degli Studi di Napoli Federico II, Portici (NA) Italy
3) Istituto di Bioscienze e BioRisorse, CNR, Portici (NA) Italy
4) Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania Luigi Vanvitelli, Caserta, Italy

6.35

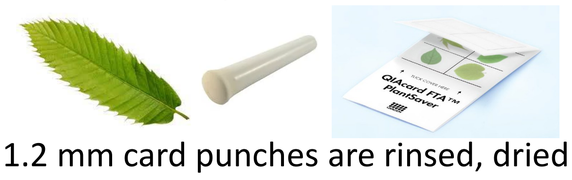
INTRODUCTION

DNA fingerprinting of fruit trees has its historical applications in the identification and classification of genetic resources, often conserved in *ex situ* and *in situ* collections. These can vary greatly in size, nature of the plant material, management and networking capabilities, and are organized in the form of living germplasm repositories, botanical gardens, or even private collections. An emerging field of application is the genetic identification of varieties for the protection of plant breeders' rights and for the management of plant nurseries. These applications are destined to expand further with the growing release of essentially derived varieties produced by the new breeding techniques (NBT), differing from the original variety by one or a few point mutations in the genome. The need to create networks between international groups responsible for the germplasm collections and for varietal identification is leading to the construction of panels of shared Single Nucleotide Polymorphisms (SNPs) to be used as "barcodes". Selected panels have already been proposed, consisting of 45, 21, and 37 SNPs, respectively for pear (*Pyrus* spp L.), apple (*Malus domestica* (Suckow) Borkh.), and sweet chestnut (*Castanea sativa* Mill.). In particular, in sweet chestnut, the availability of a fast, simple, scalable, and cost-effective workflow for genotyping small to large numbers of unknown new accessions is favoring the diffusion of the barcoding approach.

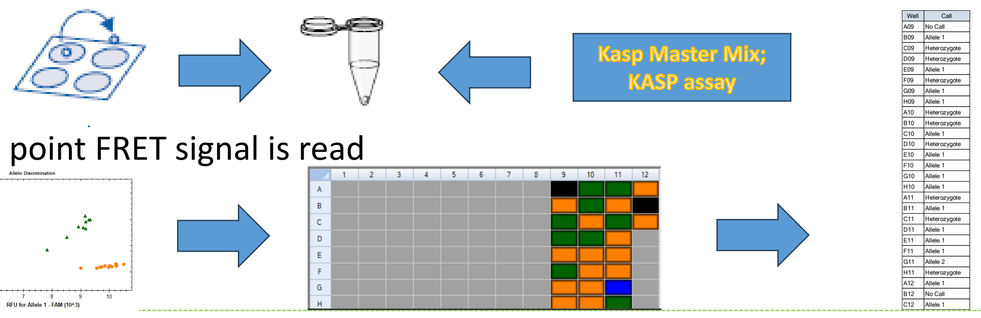
MATERIALS & METHODS

SNP DETECTION: direct Kompetitive Allele Specific PCR (dKASP)

The leaves are pestled on a Qiacard FTA plant saver and dried



1.2 mm card punches are rinsed, dried and processed directly in the PCR tube



The end point FRET signal is read

DATABASE AND DATA MINING

Results of KASP genotyping are organized in data arrays

The response to each KASP assay is expressed by a letter

Sample	A9081	A3079	A5096	A7075	A8095	B0042	B1077	B2082	B3127	B5060	C0014	C1083	C2123	C3057	C4088	C4092	C9098	D3098	D3104	E0104	E1091	E2115	F1081	F3045	G0115	G4120	G5083M	H0106	H1047	J0109	J1105	J1108	K0081	K0029	K1126	L0115	L1117	L4081
Bouche de Betizac	T	Y	M	T	W	Y	R	R	A	G	T	C	C	C	G	Y	A	G	C	C	S	Y	W	R	R	C	M	W	C	R	G	G	G	T	C	S	A	A
Lucente	A	T	A	T	T	C	G	A	A	A	T	Y	C	Y	G	C	A	G	C	Y	C		A	R	R	Y	M	W	C	A	C	G	G	C	C	G	A	A
Mercogliana	T	Y	M	K	T	T	G	G	A	A	Y	C	C	Y	G	C	M	G	C	C	S	Y	W	G	G	Y	M	W	C	A	G	K	G	Y	Y	G	T	G
Napoletana	W	T	A	T	T	Y	G	R	A	A	T	T	M	C	G	Y	A	G	C	Y	C	T	A	R	R	Y	A	A	C	A	G	G	R	Y	Y	G	A	A
Olefarella	A	C	C	T	T	Y	G	G	A	A	T	Y	C	Y	R	Y	A	G	C	C	C	Y	A	G	A	T	M	A	C	R	G	G	G	Y	C	G	A	A
Paccuta	A	T	M	T	T	Y	G	R	A	A	T	T	M	C	G	Y	A	G	C	Y	C	T	A	R	R	Y	A	W	C	A	C	K	R	Y	Y	G	W	A
San Pietro	A	T	A	T	T	Y	G	G	R	A	Y	C	C	C	G	Y	A	G	Y	C	C	C	W	G	R	Y	A	A	C	A	G	K	G	C	T	G	W	R
Tempestiva	W	T	A	K	T	Y	G	R	A	A	T	Y	M	Y	G	Y	A	G	C	Y	C	Y	W	R	G	Y	A	A	C	R	G	K	G	C	Y	S	A	A
Marzatica	A	T	M	T	T	C	G	R	A	A	Y	Y	C	C	G	C	A	T	Y	C	C	T	A	G	A	T	M	W	C	A	C	G	R	Y	C	G	W	A
Marrone di Castione	W	Y	M	T	T	C	G	R	A	R	T	C	C	C	G		A	G	C	C	S	Y	A	G	R	Y	A	W	C	A	G	G	G	Y	C	G	W	
Selvatico di Brentonico	W	Y	M	K	T	C	G	R	A	A	T	C	C	C	R	C	A	G	Y	C	S	Y	A	G	G	T	A	W	C	A	C	G	G	Y	C	G	W	A
Castagno di San Romolo	A	T	A	T	T	C	G		A	R	T	Y	C	Y	R	C	A	G	C	C	S	C	W	G	G	T	A	W	C	A	G	G	G	Y	C	G	W	A
Castagno dei Cento Cavalli	A	T	A	T	T	C	G	G	A	R	T	T	M	C	G	Y	A	T	Y	C	C	Y	W	G	G	Y	C	W	C	A	G	G	G	Y	Y	G	W	A

EXPECTED RESULTS

"VALORE IN CAMPO" PROJECT*

The re-organization of the Italian chestnut germplasm was entrusted to CREA by the Italian Ministry of Agriculture, Food Sovereignty and Forests (MASAF) and is underway by a panel of 37 SNPs, which will be expanded to 120 in a core group of reference genotypes and completed by UPOV morphological data

Sample	TG124_3	TG124_4	TG124_5	TG124_6	TG124_7	A9081	A3079	A5096	A7075	A8095	B0042	B1077	B2082	B3127	B5060	C0014	C1083	C2123	C3057	C4088	C4092	C9098	D3098	D3104	E0104	E1091	E2115	F1081	F3045	G0115	G4120	G5083M	H0106	H1047	J0109	J1105	J1108	K0081	K0029	K1126	L0115	L1117	L4081
Bouche de Betizac						T	Y	M	T	W	Y	R	R	A	G	T	C	C	C	G	Y	A	G	C	C	S	Y	W	R	R	C	M	W	C	R	G	G	G	T	C	S	A	A
Lucente						A	T	A	T	T	C	G	A	A	A	T	Y	C	Y	G	C	A	G	C	Y	C		A	R	R	Y	M	W	C	A	C	G	G	C	C	G	A	A
Mercogliana						T	Y	M	K	T	T	G	G	A	A	Y	C	C	Y	G	C	M	G	C	C	S	Y	W	G	G	Y	M	W	C	A	G	K	G	Y	Y	G	T	G
Napoletana						W	T	A	T	T	Y	G	R	A	A	T	T	M	C	G	Y	A	G	C	Y	C	T	A	R	R	Y	A	A	C	A	G	G	R	Y	Y	G	A	A
Olefarella						A	C	C	T	T	Y	G	G	A	A	T	Y	C	Y	R	Y	A	G	C	C	C	Y	A	G	A	T	M	A	C	R	G	G	G	Y	C	G	A	A
Paccuta						A	T	M	T	T	Y	G	R	A	A	T	T	M	C	G	Y	A	G	C	Y	C	T	A	R	R	Y	A	W	C	A	C	K	R	Y	Y	G	W	A
San Pietro						A	T	A	T	T	Y	G	G	R	A	Y	C	C	C	G	Y	A	G	Y	C	C	C	W	G	R	Y	A	A	C	A	G	K	G	C	T	G	W	R
Tempestiva						W	T	A	K	T	Y	G	R	A	A	T	Y	M	Y	G	Y	A	G	C	Y	C	Y	W	R	G	Y	A	A	C	R	G	K	G	C	Y	S	A	A
Marzatica						A	T	M	T	T	C	G	R	A	A	Y	Y	C	C	G	C	A	T	Y	C	C	T	A	G	A	T	M	W	C	A	C	G	R	Y	C	G	W	A
Marrone di Castione						W	Y	M	T	T	C	G	R	A	R	T	C	C	C	G		A	G	C	C	S	Y	A	G	R	Y	A	W	C	A	G	G	G	Y	C	G	W	
Selvatico di Brentonico						W	Y	M	K	T	C	G	R	A	A	T	C	C	C	R	C	A	G	Y	C	S	Y	A	G	G	T	A	W	C	A	C	G	G	Y	C	G	W	A
Castagno di San Romolo						A	T	A	T	T	C	G		A	R	T	Y	C	Y	R	C	A	G	C	C	S	C	W	G	G	T	A	W	C	A	G	G	G	Y	C	G	W	A
Castagno dei Cento Cavalli						A	T	A	T	T	C	G	G	A	R	T	T	M	C	G	Y	A	T	Y	C	C	Y	W	G	G	Y	C	W	C	A	G	G	G	Y	Y	G	W	A

MORE SNPs
The number of columns in the database will be increased

MORPHOLOGICAL DATA ON CURRRENT SEASON SHOOTS
The response to each descriptor has a coded numerical value

MORE CULTIVARS
The number of raws in the database will be increased

	TG124_3	TG124_4	TG124_5	TG124_6	TG124_7	A9081	A3079	A5096	A7075	A8095	B0042	B1077	B2082	B3127	B5060	C0014	C1083	C2123	C3057	C4088	C4092	C9098	D3098	D3104	E0104	E1091	E2115	F1081	F3045	G0115	G4120	G5083M	H0106	H1047	J0109	J1105	J1108	K0081	K0029	K1126	L0115	L1117	L4081
Cultivar 5	1	3	2	3	3	A	C	C	T	T	Y	G	G	A	A	T	Y	C	Y	R	Y	A	G	C	C	C	Y	A	G	A	T	M	A	C	R	G	G	G	Y	C	G	A	A
Cultivar 4	1	3	2	3	3	W	T	A	T	T	Y	G	R	A	A	T	T	M	C	G	Y	A	G	C	Y	C	T	A	R	R	Y	A	A	C	A	G	G	R	Y	Y	G	A	A
Cultivar 11	1	5	1	1	3	W	Y	M	K	T	C	G	R	A	A	T	C	C	C	R	C	A	G	Y	C	S	Y	A	G	G	T	A	W	C	A	C	G	G	Y	C	G	W	A
Cultivar 1	1	5	1	1	3	T	Y	M	T	W	Y	R	R	A	G	T	C	C	C	G	Y	A	G	C	C	S	Y	W	R	R	C	M	W	C	R	G	G	G	T	C	S	A	A
Cultivar 12	3	3	2	2	5	A	T	A	T	T	G	G		A	R	T	Y	C	Y	R	C	A	G	C	C	S	C	W	G	G	T	A	W	C	A	G	G	G	Y	C	G	W	A
Cultivar 6	3	3	2	2	5	A	T	T	T	Y	G	R	A	A	T	T	M	C	G	Y	A	G	C	Y	C	T	A	R	R	Y	A	W	C	A	C	K	R	Y	Y	G	W	A
Cultivar 10	3	3	2	2	5	W	Y	M	T	T	C	G	R	A	R	T	C	C	C	G		A	G	C	C	S	Y	A	G	R	Y	A	W	C	A	G	G	G	Y	C	G	W	
Cultivar 2	3	7	1	1	5	A	T	A	T	T	C	G	A	A	A	T	Y	C	Y	G	C	A	G	C	Y	C		A	R	R	Y	M	W	C	A	C	G	G	G	C	G	A	A
Cultivar 7	3	7	2	2	1	A	T	A	T	T	Y	G	G	R	A	Y	C	C	C	G	Y	A	G	Y	C	C	G	W	G	R	Y	A	A	C	A	G	K	G	C	T	G	W	R
Cultivar 3	5	7	2	3	1	T	Y	M	K	T	T	G	G	A	A	Y	C	C	Y	G	C	M	G	C	C	S	Y	W	G	G	Y	M	W	C	A	G	K	G	Y	Y	G	T	G
Cultivar 13	5	7	2	3	1	A	T	A	T	T	C	G	G	A	R	T	T	M	C	G	Y	A	T	Y	C	C	Y	W	G	G	Y	C	W	C	A	G	G	G	Y	Y	G	W	A
Cultivar 8	5	7	2	3	1	W	T	A	K	T	Y	G	R	A	A	T	Y	M	Y	G	Y	A	G	C	Y	C	Y	W	R	G	Y	A	A	C	R	G	K	G	C	Y	S	A	A
Cultivar 9	5	7	2	3	1	A	T	M	T	T	C	G	R	A	A	Y	Y	C	C	G	C	A	T	Y	C	C	T	A	G	A	T	M	W	C	A	C	G	G	G	Y	C	G	W	A

"KASTRACK" PROJECT

The KASTRACK project funded by the Campania Region is going to transfer this method for chestnut DNA fingerprinting to private laboratories.

Interactive public database

The database will be released and made interactive to guide the user to the rapid identification of scions:

1. The system requires for some morphological details about the scions:

Current season's shoot: thickness	Current season's shoot: color of upper side of stem	Current season's shoot: density of lenticels
thin ☐	yellow brown ☐	sparse ☐
medium ☐	brown ☐	medium ☐
thick ☐	red brown ☐	dense ☐

2. Based on the input and data in the database, the system suggests the top 8 best tests to verify the similarity of the scion with one of the known cultivars

Insert the results for the following tests:
A7075; C3057; C4092; E0104; E2115; F1081; K1126; L0115; L1117

3. Based on the results entered, the system will ask for further tests or give an output: **Partial string identical to: CULTIVAR 3**

Unknown sample						K											Y		C					C		Y	W														G	T	

Training for private lab will include

- Morphological data detection
- Protocols for collecting samples in the field
- Protocols for investigation by Kompetitive Allele Specific PCR (dKASP
- Reading test results
- Database query
- Data significance
- Cost evaluation

CONCLUSIONS AND PERSPECTIVES

Useful protocols are available for the rapid and cost-effective identification of chestnut cultivar. Releasing this methodology to private laboratories will help stakeholders to use this tool for their purposes. The more the technique is used, the more it will be considered conventional. However, a specific paperwork procedure will be undertaken for the technique to be recognized as evidence in the event of legal disputes or investigations on that matter. The experiences gained so far lead us to consider the conventional identification of these SNP panels as a keystone for an effective and rapid recovery of the germplasm in large and small public/private collections. To this end, an adequate subset of SNPs should be selected by mutual agreement for other species not yet explored.

* DM 667521 del 30/12/2022; CUP_J53C23000450001

66th ANNUAL CONGRESS ITALIAN SOCIETY OF AGRICULTURAL GENETICS

Bari, 5-8 September 2023