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Seed morphometry is suitable for apple-germplasm diversity-analyses

Silvia Sau[a,b], Mariano Ucchesu[a,b,*], Luca Dondini[c], Paolo De Franceschi[c], Guy D'hallewin[d], Gianluigi Bacchetta[a,b]

[a] Banca del Germoplasma della Sardegna (BG-SAR), Hortus Botanicus Karalitanus (HBK), Università degli Studi di Cagliari, Viale Sant'Ignazio da Laconi, 9-11, 09123 Cagliari, Italy
[b] Centro Conservazione Biodiversità (CCB), Dipartimento di Scienze della Vita e dell'Ambiente (DISVA), Università degli Studi di Cagliari, Viale Sant'Ignazio da Laconi, 13, 09123 Cagliari, Italy
[c] Università di Bologna, Dipartimento di Scienze Agrarie, Arboricoltura generale e coltivazioni arboree, Italy
[d] Istituto di Scienze delle Produzioni Alimentari (ISPA), Consiglio Nazionale delle Ricerche (CNR), Traversa la Crucca, 3 Loc. Baldinca, 07040 Sassari, Italy

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ABSTRACT

The main objective of this study was to evaluate the trustworthiness of seed image analysis as an approach to discriminate apple germplasm accessions. Digital images of seeds from 42 apple cultivars, acquired by a flatbed scanner, provided a phenotypic dataset with 106 morphometric variables. Stepwise Linear Discriminant Analysis (LDA) was used to examine this dataset, and the results were compared with available genetic data. The first comparison among cultivars provided a 38.8% cross-validation of correct identifications with a discriminant percentage ranging between 11.7 and 70%. In agreement with the genetic diversity analysis, the LDA could discriminate between the apples cultivars, identifying two main groups that could be further divided into additional subgroups. Based on our findings, we propose that seed image analysis is a valuable and affordable tool to investigate phenotypic diversity among a large number of apple cultivars.

1. Introduction

The Rosaceae family comprises about 3000 taxa that include many genera of great importance for human nutritional and ornamental use (Hancock, 2008). Among these, the genus *Malus* comprises about 55 species, including the domestic apple (*Malus domestica* Borkh.), one of the most economically important fruit crops grown in temperate zones (Zohary et al., 2012).

M. domestica domestication likely began in the Tian Shan Mountains in Central Asia (Harris et al., 2002; Cornille et al., 2012). This area contains multiple crop wild relatives (CWR) of domestic apples, such as *M. sieversii* (Ledeb.) M. Roem., which is fully interfertile with *M. domestica* (Zohary et al., 2012). Other important species of wild apples have genetically contributed to domestic apple, including *M. orientalis* Uglitzk. ex Juz., with a distribution range identified in the Caucasus, and *M. sylvestris* (L.) Mill., distributed primarily in Europe (Cornille et al., 2014).

Molecular marker studies of wild and cultivated apples have confirmed the diffusion of apple across the silk road from Central Asia, passing through Turkey towards Europe (Cornille et al., 2013a, 2013b, 2012; Velasco et al., 2010; Harris et al., 2002). Nevertheless, the origin of apple domestication remains partially unclear because of interfertility and self-incompatibility of *Malus* species, which can hybridize, thereby generating highly variable progenies (Zohary et al., 2012; Cornille et al., 2012; Velasco et al., 2010). There are over 10,000 cultivars of apples worldwide, showing huge variability in their traits, especially pomological features such as the fruit size, skin colour and taste (Cornille et al., 2014, 2012; Harris et al., 2002).

Today, the number of modern commercial cultivars has been reduced because of clonal selection and breeding programs, which used a small number of genotypes (Hokanson et al., 2001; Noiton and Alspach, 1996). For this reason, to maintain the greatest variation of alleles that can be exploited in breeding programs, several researchers have recommended protecting and preserve CWRs and old apple cultivars (Liang et al., 2015; Way et al., 1990; Nnadozie et al., 2003).

Several genetic studies were conducted to investigate the origins of apple domestication and genetic diversity within the species or within the local germplasm (Urrestarazu et al., 2016, 2012; Cornille et al., 2012; Liang et al., 2015; Velasco et al., 2010).

Liang et al. (2015), by simple sequence repeats (SSRs) analysis, described the genetic diversity within a large number of apple cultivars (belonging mainly to the Italian peninsula), with the goal of identifying

* Corresponding author at: Banca del Germoplasma della Sardegna (BG-SAR), Hortus Botanicus Karalitanus (HBK), Università degli Studi di Cagliari, Viale Sant'Ignazio da Laconi, 9-11, 09123 Cagliari, Italy.
E-mail address: marianoucchesu@gmail.com (M. Ucchesu).

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Table 1
Cultivars of *M. domestica* analysed in this study.

Code	Origin	Cultivar name	N° Seeds	Code	Origin	Cultivar name	N° Seeds
C1	ITA	ABBONDANZA	81	C22	ITA	MELA GIALLA 1	105
C2	TURK	AMASYA	118	C23	ITA	MELA ROZZA	112
C3	ITA	ANNURCA	80	C24	ITA	MELA TOSTA	60
C4	ITA	APPIA (RT)	93	C25	ITA	MELO FERRO (PD)	34
C5	ITA	APPIONA	80	C26	ITA	OXIU	94
C6	NLD	BELLA DI BOSKOOP	17	C27	ITA	PAOLUCCIA (VT)	100
C7	ITA	BELLA DEL GIARDINO	100	C28	ITA	PARADISA	69
C8	ITA	BOURAS	86	C29	ITA	PUMA TENERELLA	97
C9	ITA	CADDINA	54	C30	FRA	RAMBOUR FRANK	96
C10	ITA	CAVICCHIO	72	C31	USA	RED CHIEF	86
C11	GER	CLIVIA	99	C32	NLD	RENETTA ANANAS	91
C12	ITA	DURELLO	97	C33	ITA	RENETTA DI CHAMPAGNE	99
C13	ITA	EPPIA	57	C34	FRA	REINETTE FRANCHE (M.REGINA)	64
C14	ITA	FIOR DI CASSIA	77	C35	ITA	ROSA D'OSTA	35
C15	ITA	FRANCESCA (MI)	121	C36	ITA	RUNSE'	133
C16	ITA	GELATA	92	C37	ITA	SANT'AGOSTINO	76
C17	ITA/FRA	GRENOBLE (TO)	86	C38	ITA	SEL IDICE 3	51
C18	ITA	LIMONCELLA	113	C39	ITA	SEL IDICE 4	100
C19	ITA	LIMONCELLA URIDDU	89	C40	ITA	VERGINELLA	95
C20	ITA	LOSA D'GIAVENO	93	C41	ITA	VIGNONE	75
C21	ITA	MARCON (TN)	89	C42	ITA	PUMA OLIO	95

synonymy and homonymy (which are extremely difficult to detect through phenotypic traits) and exploring the genetic structure detectable in this large asset of accessions.

Many recent papers testify the importance of the seed image and Linear Discriminant Analysis (LDA) to characterize and investigated the cultivated species such as *Vitis vinifera* L. spp. *vinifera*, *Olea europaea* L., *Cucumis melo* L. and *Prunus domestica* L., (Orrù et al., 2015, 2013, 2012; Ucchesu et al., 2017, 2016, 2015; Sabato et al., 2015; Piras et al., 2016; Sarigu et al., 2017).

The main objectives of this work were:

(1) to build a database of seed morphological variables of apple cultivars, suitable for cultivar characterization;
(2) to assess the phenotypic diversity of apples by morphological seed image analysis techniques and by LDA;
(3) to compare our seed image analysis data with a genetic study previously conducted on the same cultivars (Liang et al., 2015).

2. Materials and methods

2.1. Apple germplasm cultivars

In this work, we have investigated 42 apple cultivars previously subjected to genetic analysis by Liang et al. (2015) (Table 1).

The all fruits were harvested in summer from the field catalogue of Cadriano Experimental Station of the Department of Agricultural Sciences (University of Bologna) at full ripening. After removing the flesh, seeds were cleaned, washed and naturally air-dried in the laboratory of the Sardinian Germplasm Bank (BG-SAR), University of Cagliari.

To ensure the highest morphological variability among seeds and to compare morphometric results with genetic data, 10 fruits of each cultivar were harvested from the same tree previously used for the genetic analysis. To facilitate the presentation of results and sample grouping, each cultivar has been coded (Table 1).

2.2. Seeds image analysis

Digital image analysis is an innovative method of recent use that allows a high number of morphometric features of the seeds (Keefe and Draper, 1988).

This method gives several advantages such as low-cost analysis, non-destruction of the sample, analytical speed compared to conventional methods, even in the presence of a large amount of data and the ability to standardize the process making it interactive and easy to use (Chitra et al., 2016; Sandeep et al., 2013; Nikam and Kakatkar, 2013).

Moreover, this methodology can be applied to a large field of investigations such as the agronomic one, for example for identifying new cultivars or identifying possible synonyms and homonyms groups (Orrù et al., 2015, 2013, 2012; Ucchesu et al., 2017, 2016, 2015; Sabato et al., 2015; Piras et al., 2016; Sarigu et al., 2017).

The digital images of all seeds were acquired using a flatbed scanner (Epson Perfection V550), with a digital resolution of 800 dpi for a scanning area not exceeding 1024 × 1024 pixels (Bacchetta et al., 2008). The images were processed and analysed using the open source software ImageJ v. 1.49. The Particles8 plugin (Landini, 2006) was used to measure 26 seed morphometric variables (Table 2, Fig. 1).

Table 2
Morphometric variables measured on each apple seed, calculated according to the Particles8 plugin software for ImageJ v. 1.49.

Parameter	Description
Perim	Perimeter, calculated from the centres of the boundary pixels
Area	Area inside the polygon defined by the perimeter
Pixels	Number of pixels forming the endocarp image
MinR	Radius of the inscribed circle centred at the middle of mass
MaxR	Radius of the enclosing circle centred at the middle of mass
Feret	Largest axis length
Breadth	Largest axis perpendicular to the Feret
CHull	Convex hull or convex polygon calculated from pixel centres
CArea	Area of the convex hull polygon
MBCRadius	Radius of the minimal bounding circle
AspRatio	Aspect ratio = Feret/Breadth
Circ	Circularity = $4{\cdot}\pi{\cdot}Area/Perimeter^2$
Roundness	Roundness = $4{\cdot}Area/(\pi{\cdot}Feret^2)$
ArEquivD	Area equivalent diameter = $\sqrt{((4/\pi){\cdot}Area)}$
PerEquivD	Perimeter equivalent diameter = $Area/\pi$
EquivEllAr	Equivalent ellipse area = $(\pi{\cdot}Feret{\cdot}Breadth)/4$
Compactness	Compactness = $\sqrt{((4/\pi){\cdot}Area)}/Feret$
Solidity	Solidity = Area/Convex_Area
Concavity	Concavity = Convex_Area-Area
Convexity	Convexity = Convex_hull/Perimeter
Shape	Shape = $Perimeter^2/Area$
RFactor	RFactor = Convex_Hull /$(Feret{\cdot}\pi)$
ModRatio	Modification ratio = $(2{\cdot}MinR)/Feret$
Sphericity	Sphericity = MinR/MaxR
ArBBox	Area of the bounding box along the feret diameter = $Feret{\cdot}Breadth$
Rectang	Rectangularity = Area/ArBBox