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Micromorphological, Biochemical, and Genetic Characterization of two Ancient, Late-bearing Apple Varieties

A. Minnocci, P. Iacopini, F. Martinelli and L. Sebastiani
(Scuola Superiore Sant'Anna, Pisa, Italy)

Summary

Two ancient, late-bearing apple varieties (cv. 'Diacciata' and 'Limoncella') were characterized using micromorphological, genetic and biochemical approaches, and by comparison with two commercial varieties, 'Gala' and 'Golden Delicious'. There were significant differences between the two varieties. Cryo scanning electron microscopy of frozen-hydrated leaves showed strong differences in epidermal cells, epicuticular waxes, stomatal structure, density, and leaf lamina internal tissues. We compared the two varieties at 21 SSR alleles; the mean heterozygosity and power of discrimination were 0.76 and 0.60, respectively. SSR loci GD 147 and GD 162 were sufficient to discriminate the two late-bearing varieties. 'Limoncella' had the most soluble solids, total phenolics, total flavonoids, epicatechin and chlorogenic acid. This cultivar also had higher acidity and peroxynitrite scavenging capacity. 'Diacciata' had the highest average weight, flesh firmness, catechin and caffeic acid content. These two ancient, late-bearing varieties demonstrate to be well adapted to the Mediterranean climate, in which they produce high-quality fruit with good organoleptic properties and high concentrations of desirable phytochemicals. Therefore, they may be of interest as a source of genes for breeding programs in Mediterranean basin.

Key words. antioxidant activity – cryo SEM – 'Diacciata' – leaf – 'Limoncella' – fruit – *Malus domestica* – SSR

Introduction

Loss of genetic resources is a chronic problem for breeders working on genetic improvement of fruit trees. Breeding programs have sought out and described high-value local genotypes that are maintained in collection fields (Sansavini and Watkins 1991). Two late-bearing apple (*Malus* × *domestica* Borkh.) cultivars have been grown since Ancient Roman times in the Apennine Mountains of south-central Italy (Neri 2004). They are called 'Diacciata' and 'Limoncella' close to Lucca (Tuscany), but synonyms are used in other areas: 'Ghiacciola' and 'Lemonella' in Tuscan Apennine; 'Ghiaccio' and 'Limoncella' in Basilicata, Molise and Campania; 'Gelata' in the whole south-central Italy. 'Diacciata' bears a round, medium sized fruit with a green or green-yellow epicarp and a white, juicy, sweet, and fragrant pulp. The commercial use is for fresh consumption. 'Limoncella' is particularly vigorous and productive on high hills or medium mountains. The fruit is small, elongated, and conical, develops a yellow skin and a citrus flavour that lasts until the end of spring. 'Limoncella' is produced both for fresh consumption and for juice. Both varieties have superb flavor, are resistant to diseases, and they will maintain their particular organoleptic properties through the winter without cold storage (Neri 2004).

Traditional methods of cultivar characterization, using agronomic and morphological traits, have the disadvantage that trait expression is strongly affected by environmental conditions, tree age, training systems, and the phenological stage of the plants. For this reason, such methods are often replaced or complemented with other methods, such as micromorphological traits (Viti et al. 2000; Curry 2005) or DNA marker systems such as RFLP, RAPD, AFLP, SNP or SSR (microsatellites). Among molecular methods, SSRs have become the markers of choice in several plant species because of their abundance, high degree of polymorphism, high reproducibility, co-dominant inheritance, and suitability for automation. Many SSR markers have been developed in apple (Hokanson et al. 1998; Guarino et al. 2006).

Biochemical traits are also useful to distinguish apple cultivars. Apple fruits contain several major classes of phenolic compounds in the peel and flesh: flavan-3-ol monomers (catechin and epicatechin), flavan-3-ol polymers (procyanidins), dihydrochalcones (phloretin glycosides), flavonols (quercetin glycosides), hydroxycinnamic acids (chlorogenic and caffeic acid) and, in red-skinned cultivars, anthocyanins (Alonso-Salces et al. 2001). Apple polyphenols have reported antiallergenic and anti-carcinogenic in vitro properties and inhibitory activity against some enzymes and receptors in vivo (Shoji et al. 2004).

There are several methods to evaluate the antiradical activity of apple extracts and pure compounds, with their efficacy depending on the exact environmental conditions and procedures used. In vitro assays for free radical scavenging capacity are commonly based on inactivation of stable synthetic radicals such as 2,2-diphenyl-1-picrylhydrazyl (DPPH, Blois 1958). Another interesting ap-

proach uses peroxynitrite ($ONOO^-$) scavenging capacity. Peroxynitrite is a strong and versatile oxidant produced by reacting nitric oxide and superoxide. Its importance in biological systems is based on its powerful ability to react with almost all biomolecule classes (Virág et al. 2003), thus contributing to the onset and maintenance of pathologies such as atherosclerosis, neurodegenerative diseases and cardiovascular disorders (Wattanapitayakul et al. 2000). Fruit compounds that scavenge deleterious radicals and other compounds can contribute to the health maintenance or healing (Prieto et al. 2007).

The objective of this study was to perform a detailed micromorphological, genetic and biochemical characterization of the two ancient late-bearing cultivars 'Diacciata' and 'Limoncella'. We used cryo-scanning electron microscopy to define leaf lamina characteristics such as upper surface, stomata structure and density, and internal tissue structure. Genetic differences, the phenolic composition and antioxidant activity of the fruit were also determined. Biochemical properties are fundamental to understand the nutraceutical potential of these two ancient apple varieties and their suitability as a source of genes for breeding programs.

Materials and Methods

Chemicals and reagents

All chemicals were of analytical or higher grade and were purchased from Sigma Aldrich (Milan, Italy). Aqueous solutions were prepared using ultra-pure water purified by Milli-Q System (Millipore, Milan, Italy).

Plant material and fruit characteristics

Two ancient, late bearing apple (*Malus* × *domestica* Borkh.) cultivars ('Diacciata' and 'Limoncella') were compared with two modern commercial cultivars ('Gala' and 'Golden Delicious'). Samples were collected in 2007 from certified apple plants in a nursery specializing in ancient cultivars located in Lucca (Tuscany, Italy). Green, fully expanded leaves and 6 mm leaf discs were sampled in the spring and fruits were collected at their commercial maturity. Three replicates of four fruits were collected for each variety. For qualitative description, physicochemical parameters such as fresh weight (g), soluble solids (°Brix), firmness (kg cm^{-2}), titrable acidity (g malic acid L^{-1}), and pH were determined at the tipical harvest time (the end of November for 'Diacciata' and 'Limoncella', late-ripening varieties; the end of September for the commercial 'Gala' and 'Golden Delicious'). Flesh firmness was measured on opposite sides of each fruit with a penetrometer (Effegi, Milan, Italy). Juice pressed from the whole fruit was used to measure soluble solids with a hand-held refractometer (Sper Scientific, AZ, USA) and titratable acidity by titrating 10 mL juice with 0.1 N NaOH to pH 8.1 using 1 % phenolphthalein as an indicator.

Micromorphological analysis

Immediately after sampling, ten leaf discs from each cultivar were cryo-fixed in liquid nitrogen (−196 °C) for surface and freeze-fractured observations. Samples were maintained in their frozen-hydrated state until moved to a cryo-preparation chamber (SCU 020 Bal-Tech, Liechtenstein) dedicated to a Scanning Electronic Microscope (Philips SEM 515), where they were surface etched at −80 °C, freeze-fractured (−120 °C) to expose the internal structures, and sputtered with a 10 nm gold and analysed in the Cryo-SEM chamber at −150 °C (Marchi et al. 2008). Stomatal density was measured on the lower leaf surface on a scanning area of 441.4 × 331.0 μm (n = 5) and results were expressed as stomata mm^{-2}. The digital images were used to determine the whole leaf and tissue layer thicknesses (upper and lower epidermis, palisade and spongy parenchyma).

Molecular characterization

Green, fully-expanded leaves were sampled from three different plants, washed carefully with sterile water, and stored immediately at −80 °C. DNA extraction was performed with the DNeasy Plant MiniKit (Qiagen) according to manufacturer's instructions. DNA quantification was carried out using agarose gel electrophoresis (1 %). For SSR analysis, 20 ng DNA was used. The SSR markers GD 96, GD 100, GD 142, GD 147, and GD 162, specific for the apple genome, were chosen because they showed a high power in discriminating different genotypes within the same apple species (Hokanson et al. 1998). Amplification products were analyzed on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, USA). Microsatellites were evaluated by expected heterozygosity (H) (Nei 1973) according to the formula $H = 1 - \Sigma\, p_i^2$ where p_i is the frequency of i^{th} allele at the studied SSR locus. Discrimination power (D) was calculated according to the formula as above, where p_i represents the frequency of the i^{th} genotype.

Biochemical analysis

Phenolics were extracted from 60 g fresh peel and pulp using 80 % aqueous methanol up to a final volume of 200 ml and filtered through a 0.45 μm cellulose filter (Millipore, Milan, Italy) before HPLC analysis. The HPLC system consisted of a Dionex P 680 Pump coupled with a Dionex UVD 170U/340U UV/VIS detector (Dionex, Germany). Separation was performed on a reversed-phase C18 column (Acclaim 120, 5 μm, 4.6 × 250 mm, Dionex) coupled with a C18 guard cartridge (Acclaim 120, 5 μm, 4.3 × 10 mm, Dionex) at room temperature. Elution conditions were as follows: solvent A: water; solvent B: methanol; solvent C: acetic acid; flow rate 0.8 ml min^{-1}; 20 μl injection volume. Detection was at 280 nm for catechin and epicatechin, 320 nm for chlorogenic acid and caffeic acid, and 360 nm for rutin. Identification was based on comparing retention times and UV-Vis spectral data to those of reference standard compounds. Quantification was accomplished using external calibration with pure standards ($R^2 = 0.999$). The elution program was: B 5 % and C 3 % as initial conditions, B to 55 % in 40 min, B to 100 % in 10 min, hold for 10 min, B to 5 % in 5 min, rinse for 5 min. Data were processed using the software Chromeleon 6.5 (Dionex, Germany).

Total phenolic content (TPC) as mg gallic acid equivalents (GAE) per 100 g of fresh material was measured as described (Singleton and Rossi 1965). Flavonoid concentration as mg catechin equivalents (CE) per 100 g fresh material was determined using a colorimetric assay (Zhishen et al. 1999). Inhibition of tyrosine nitration by peroxyni-

trite was based on the determination, by reversed-phase HPLC-UV analysis, of 3-NT formation by the reaction between free tyrosine and peroxynitrite at physiological pH (IACOPINI et al. 2008). Peroxynitrite was synthesized according to the method of BECKMAN et al. (1994). The concentration of the stock solution was evaluated immediately before use by measuring the absorbance at 302 nm (ε_{ONOO^-} = 1670 M^{-1} cm^{-1}). The peroxynitrite scavenging ability of the extracts was expressed as Trolox Equivalents (TE) in 100 g fresh material. DPPH radical scavenging activity was evaluated using DPPH, a stable free radical compound with a characteristic absorption at 515 nm (BRAND-WILLIAMS et al. 1995). To find the time required to achieve the steady state (T_{ss}), absorbance at 515 nm (Abs_{515}) was measured at different time intervals on a UV-VIS spectrophotometer (Lambda 25, Perkin Elmer, Italy) until the reaction reached a plateau. Then, the decrease in absorbance was recorded when the reaction reached the steady state. The initial concentration of DPPH was determined for every experiment by reference to a standard curve. The DPPH radical-scavenging effect was expressed as percentage reduction (%R) of the initial DPPH absorption:

$$\%R \text{ of the initial DPPH absorption} = [(A_{DPPH}(t) - A_{sample}(t))/A_{DPPH}(t)] \times 100$$

where $A_{DPPH}(t)$ is the absorbance of DPPH at time t and $A_{sample}(t)$ is the absorbance of the sample at the same time t. HPLC elution conditions for the detection of 3-NT was as follows: 20 mM phosphate buffer (pH 3.2)/methanol 92:8 v/v; flow rate 1 ml min^{-1} in isocratic mode, 20 µl injection volume; UV detection at 356 nm.

Statistical analysis

Results presented in tables and graphs were reported as means ± standard deviation (SD), with 10 and 3 replications, respectively, for micromorphological and biochemical parameters. Data were subjected to t-test or ANOVA and differences within mean values were tested after ANOVA by post-hoc comparison test (Tukey's HSD) at P = 0.05.

Results and Discussion

Leaf micromorphological and fruit physicochemical characteristics

The upper leaf surfaces (Fig. 1A and 1B) of the two ancient cultivars were quite different. The epidermal cells of 'Diacciata' were almost twice as big as those of 'Limoncella' (565.8 ± 118.6 µm² and 275.0 ± 37.9 µm², respectively). 'Diacciata' had long and filamentous epicuticular wax with an average length of 1.9 µm (Fig. 1A, arrow), but that of 'Limoncella' was spherical with an average diameter of 1.1 µm (Fig. 1B, arrow). The lower leaf surfaces of the two cultivars were also different (Fig. 1C, 1D). *Stomata* of 'Diacciata' were slightly shorter and wider (32.1 ± 1.6 µm × 22.5 ± 0.3 µm) than those of 'Limoncella' (33.5 ± 0.4 µm × 18.5 ± 1.2 µm). Only the difference in width was statistically significant. The stomatal density of fully-expanded leaves was 220.1 stomata mm^{-2} for 'Diacciata' and 334.1 stomata mm^{-2} for 'Limoncella' (P < 0.001).

Total leaf lamina thickness was statistically greater for 'Limoncella' than 'Diacciata' (Table 1). These differences were principally due to the palisade and spongy parenchyma tissues, not epidermis (Fig. 1E, 1F). This is consistent with other published findings (MARCHI et al. 2008).

'Diacciata' fruit weight at harvest was four times greater than 'Limoncella' and approximately, twice that of the commercial varieties (Table 2). Moreover, the ancient varieties had firmer flesh and greater soluble solids. 'Limoncella', in particular, had very high soluble solids (28.2 °Brix), the highest acidity, and the lowest pH. This high sugar content is an interesting feature for an apple cultivar, both for its taste and for its effect on storage-life, as sugars are a cryoprotectant.

Molecular characterization

The cultivars were genetically analyzed using five previously published SSR markers known to discriminate among apple cultivars (HOKANSON et al. 1998). All five SSR markers produced polymorphic amplification products in our four samples (Table 3). The total number of alleles amplified by the five primers was 21. The average number of alleles per locus was four, ranging from a minimum of three for GD62 to a maximum of five for GD96 and GD162. The gene diversity or heterozygosity (H) ranged from 0.63 to 0.83, with an average of 0.76. The number of genotypes ranged from three for GD147 to five for GD162. The power of discrimination (D) ranged from 0.5 to 0.66, with a mean of 0.6. Considering the small number of cultivars analyzed, H and D were similar to those showed in previous work using the same primers (HOKANSON et al. 1998) and other markers (GUARINO et al. 2006). All five SSRs primers produced PCR amplicons that were polymorphic and easily scorable in all four cultivars as from previously published work (HOKANSON et al. 1998). Two SSR locis, GD 147 and GD 162, were

Table 1. Internal structures of fully-expanded leaf lamina of late-bearing apple cultivars.

Cultivar	Total thickness	Upper epidermis	Palisade tissue	Spongy tissue	Lower epidermis
'Diacciata'	242.35 ± 1.85 b⁺	14.70 ± 0.54	118.26 ± 0.30 a	100.97 ± 2.36 b	8.12 ± 0.88 b
'Limoncella'	251.82 ± 0.62 a	14.37 ± 1.89	107.33 ± 1.75 b	120.46 ± 1.13 a	9.66 ± 1.14 a
P values	***	NS	***	***	**

*** P < 0.001, ** P < 0.01. NS not significant.
⁺ The values are the means ± SD (n = 10) (µm). Within each column different letters indicate statistically different values according to t-test (Tukey's HSD).

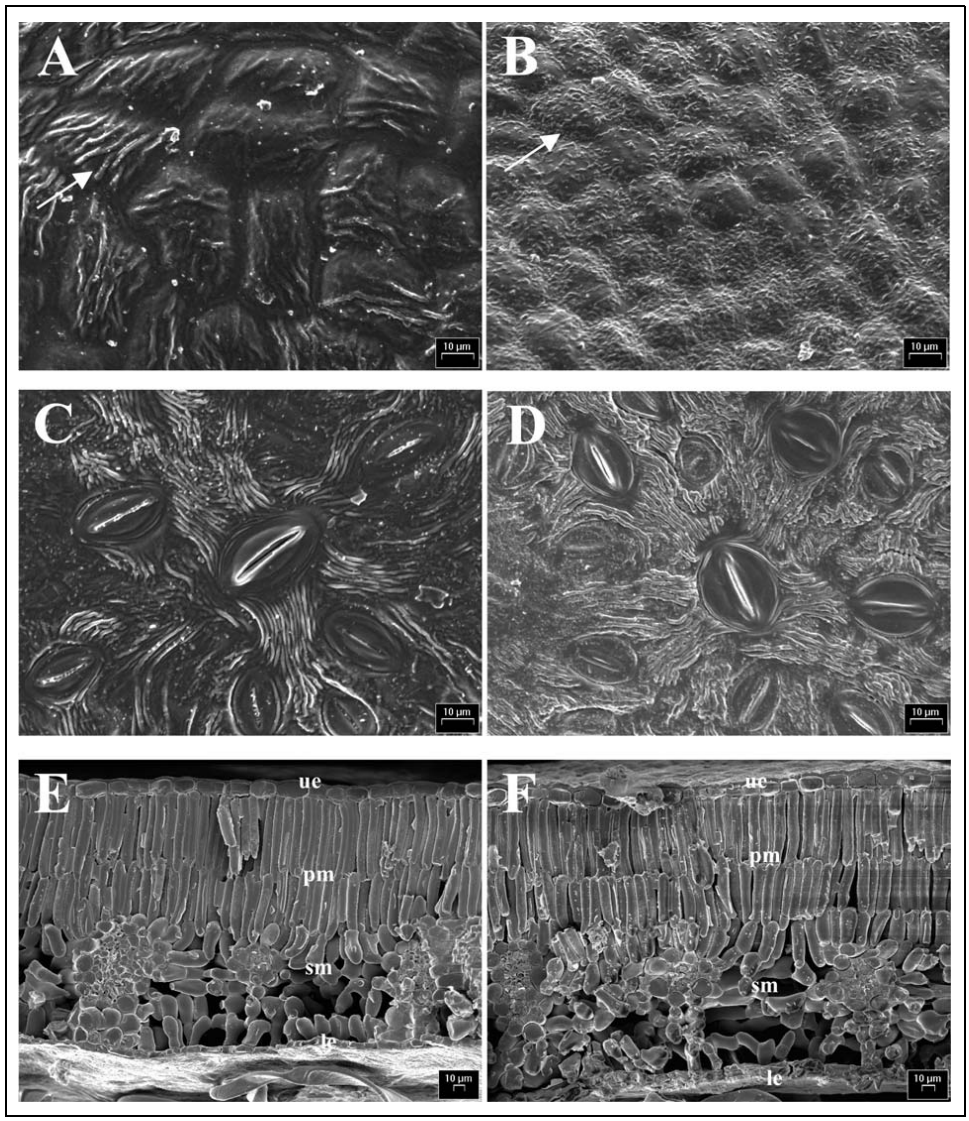


Fig. 1. Cryo-SEM images of frozen-hydrated leaves. Upper leaf surface of (A) cv. 'Diacciata' and (B) cv. 'Limoncella' (white arrows = epicuticular waxes). Stomata on lower leaf surface of cv. 'Diacciata' (C) and cv. 'Limoncella' (D). Transverse freeze-fractures of leaf lamina showing internal tissues structure of cv. 'Diacciata' (E) and cv. 'Limoncella' (F) (ue = upper epidermis; pm = palisade mesophyll; sm = spongy mesophyll; le = lower epidermis).

Table 2. Apple fruit samples: physicochemical parameters at harvest time, year 2007.

Cultivar	Average weight (g)	Flesh firmness (kg cm^{-2})	Soluble solids (°Brix)	Acidity (g L^{-1})	pH
'Diacciata'	260.2 ± 0.9^{+} a	7.7 ± 0.1 a	19.1 ± 0.1 b	4.3 ± 0.3 b	3.8 ± 0.01 b
'Limoncella'	64.1 ± 0.1 d	6.3 ± 0.1 b	28.2 ± 0.2 a	5.3 ± 0.1 a	3.3 ± 0.01 d
'Gala'	167.2 ± 24.3 b	5.2 ± 0.7 c	11.9 ± 0.1 d	2.8 ± 0.1 c	4.2 ± 0.05 a
'Golden Delicious'	120.2 ± 19.8 c	5.7 ± 0.3 bc	13.7 ± 0.5 c	4.2 ± 0.4 b	3.6 ± 0.10 c
P ANOVA	***	***	***	***	***

*** $P < 0.001$.
$^{+}$ Values are the means ± SD (n = 3). Within each column different letters indicate statistically different values according to t-test (Tukey's HSD).

sufficient to discriminate between the two ancient apple cultivars. The additional three SSR loci (GD 96, GD 100, and GD 142) also distinguished them from the two commercial cultivars. Guarino et al. (2006) easily discriminated several apple cultivars, including the local accession of 'Limoncella', using SSR-based fingerprinting. No SSR-based profiles for 'Diacciata' have been previously published.

Polyphenolic composition

The total phenolic and total flavonoid content of the whole fruit extracts was evaluated (Table 4). The total phenolics and flavonoids concentrations in 'Limoncella' were particularly high, around twice that of 'Diacciata' and almost three times higher than 'Gala'. A similar concentration of total phenolics and flavonoids was found in 'Limoncella' by D'Abrosca et al. (2007), while no data about phenolic content and antioxidant activity of 'Diacciata' have previously been published.

Concentrations of the principal phenol constituents of the apple extracts are reported (Table 5). On the basis of published data on other apple varieties (Escarpa and González 1998), catechin, epicatechin, rutin, chlorogenic, and caffeic acid were selected to be determined and quantified. All four apple cultivars contained high amounts of epicatechin and chlorogenic acid, the averages being 4.8 ± 0.3 and 17.7 ± 1.4 mg 100 g FW^{-1}, respectively. 'Limoncella', had statistically more epicatechin than the other cultivars. 'Diacciata' had the least chlorogenic acid. Both ancient varieties had more catechin than the modern commercial ones, while 'Limoncella' had the least caffeic acid and 'Golden Delicious' and 'Diacciata' the most. The rutin content was very low, averaging 0.52 ± 0.06 mg 100 g FW^{-1}, but 'Gala' had the most.

Antioxidant activity

The antioxidant power of the methanol extract each cultivar was determined using two different *in vitro* assays: scavenging of the stable, synthetic DPPH radical and inhibition of tyrosine nitration by peroxynitrite. All extracts were significantly active as antioxidant agents.

The 'Limoncella' extract had the most DPPH radical-scavenging capacity, and 'Golden Delicious' the least, as previously reported (Drogoudia et al. 2008). While 'Limoncella' reduced DPPH by 91.2 % after just 5 min, 'Golden Delicious' reduced it by only 50.6 % in the same time. The 'Gala' extract was statistically similar to 'Golden Delicious' at 5 min, but was statistically equivalent to 'Diacciata' after 35 min and to both ancient varieties after 65 min (Fig. 2).

All the apple extracts analyzed had high scavenging capacity towards the radicals generated by peroxynitrite and were able to reduce the formation of 3-NT (Table 4). 'Limoncella' had the highest activity (35.1 ± 2.5 TE 100 g

Table 3. SSR fragment size (bp) of the apple cultivars studied and some SSR parameters (number of alleles, size range (bp), heterozygosity (H), and power of discrimination (D).

Cultivar	GD 96	GD 100	GD 142	GD 147	GD 162
'Diacciata'	n.d.	n.d.	n.d.	135–137	220–229
'Limoncella'	161–174	224–227	133–149	135–139	211–236
'Gala'	150–178	225–237	139–141	n.d.	211–232
'Golden Delicious'	170–178	209–225	n.d.	n.d.	n.d.
SSR parameters					
N° of alleles	5	4	4	3	5
Size range	150–178	209–237	133–149	135–139	211–236
H	0.83	0.80	0.75	0.63	0.78
D	0.67	0.67	0.50	0.50	0.67

n.d. not detected.

Table 4. Total phenolic, flavonoid content and inhibition of tyrosine nitration by peroxynitrite capacity in apple fruits.

Cultivar	Total phenolics (GAE 100 g FW^{-1})	Total flavonoids (CE 100 g FW^{-1})	$ONOO^-$ scavenging capacity (TE 100 g FW^{-1})
'Diacciata'	70.3 ± 5.22 c[+]	50.1 ± 3.03 c	21.3 ± 2.3 b
'Limoncella'	135.9 ± 7.65 a	118.5 ± 9.42 a	35.1 ± 2.5 a
'Gala'	56.0 ± 3.99 c	40.3 ± 1.23 d	18.5 ± 0.7 b
'Golden Delicious'	104.3 ± 11.20 b	65.0 ± 7.46 b	17.7 ± 0.1 b
P ANOVA	***	***	***

*** $P < 0.001$.

[+] Values are the means ± SD (n = 3). Within each column different letters indicate statistically different values according to t-test (Tukey's HSD).

GAE, gallic acid equivalents; CE, catechin equivalents; TE, Trolox equivalents.

Table 5. Catechin, epicatechin, rutin, chlorogenic acid, and caffeic acid content in apple fruits.

Cultivar	Phenolic content (mg 100 g FW^{-1})				
	Catechin	Epicatechin	Chlorogenic acid	Caffeic acid	Rutin
'Diacciata'	4.54 ± 0.35 a+	3.73 ± 0.26 b	7.53 ± 0.34 c	1.22 ± 0.14 ab	0.39 ± 0.04 b
'Limoncella'	4.42 ± 0.01 a	6.59 ± 0.41 a	36.27 ± 2.64 a	0.47 ± 0.04 c	0.34 ± 0.02 b
'Gala'	1.09 ± 0.08 b	4.25 ± 0.04 b	12.25 ± 0.08 b	1.06 ± 0.01 b	0.99 ± 0.05 a
'Golden Delicious'	0.70 ± 0.07 b	4.68 ± 0.59 b	14.86 ± 2.40 b	1.43 ± 0.10 a	0.35 ± 0.15 b
P ANOVA	***	***	***	***	***

*** P < 0.001.
+ Values are the means ± SD (n = 3). Within each column different letters indicate statistically different values according to *post-hoc* comparison (Tukey's HSD) at P = 0.05.

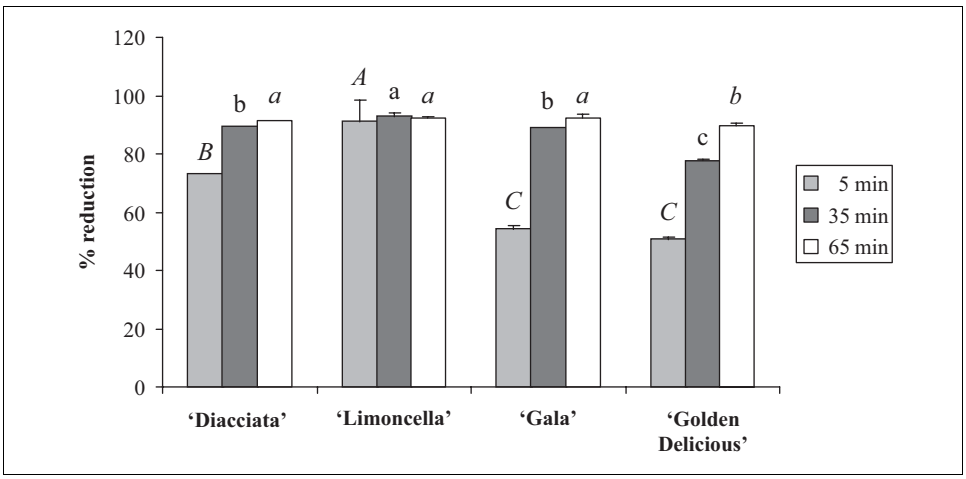


Fig. 2. Antioxidant activity: changes in DPPH radical scavenging activity after different times of reaction (5, 35, 65 min). Different letters indicate statistically different values according to post-hoc comparison (Tukey's HSD) at P = 0.05.

FW^{-1}). 'Diacciata' and the two commercial varieties were statistically equivalent, the average being 19.2 ± 1.0 TE 100 g FW^{-1}, slightly more than half of the 'Limoncella' activity.

Significant correlations between antioxidant activity and phenolic content were found, as previously reported for 'Golden Delicious' and new varieties derived from it (Wojdylo et al. 2008). Total phenols and total flavonoids also correlated positively with the capacity to inhibit tyrosine nitration by peroxynitrite, the R^2 being respectively 0.555 and 0.829. DPPH scavenging capacity correlated significantly with total flavonoids after 5 min of reaction (R^2 = 0.556), while there was no significant correlation with total phenolic content.

Conclusions

The climate of south-central Italy is ideal for late-ripening apple varieties, that increase size in autumn, unlike earlier commercial cultivars that develop during the summer when drought reduces vegetative growth. These traditional varieties, with very firm flesh, high acidity, superb flavour and high disease resistance (Neri 2004), are well adapted to the Mediterranean climate and thus potentially valuable in other regions of the Mediterranean basin.

The two ancient, late-ripening varieties 'Limoncella' and 'Diacciata' were significantly different from each other and from the two commercial varieties 'Gala' and 'Golden Delicious' for all parameters investigated. 'Limoncella' had the highest soluble solids, acidity, total phenolics, total flavonoids, catechin, epicatechin, and chlorogenic acid and the highest peroxynitrite scavenging capacity. 'Diacciata' had the highest average fruit weight, the firmest flesh, and the most catechin and caffeic acid.

The unique characteristics of these ancient apple varieties demonstrate the importance of maintaining traditional cultivars, even those of minor economic impact, since they are of high quality and may be a good source of phytochemicals, bioactive compounds with important protective properties. These apple cultivars are also an important source of genes for apple breeding programs which product added value apple cultivars, to hit growing consumer demands for healthier and better quality foods.

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Addresses of authors: Antonio Minnocci (corresponding author), Patrizia Iacopini, Federico Martinelli and Luca Sebastiani, BioLabs, Scuola Superiore Sant'Anna, Piazza Martiri della Libertà 33, I-56127 Pisa, Italy, e-mail: antonio.minnocci@sssup.it.