Molecular Characterization of Apricot Varieties Included in the “Albicocca Vesuviana” PGI Regulation

R. Rao1, M. Bencivenni1, G. Corrado1, B. Basile2 and M. Forlani2
1Dipartimento di Scienze del Suolo, della Pianta, dell’Ambiente e delle Produzioni Animali
2Dipartimento di Arboricoltura, Botanica e Patologia Vegetale
Università degli Studi di Napoli Federico I, Portici
Italy

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Abstract

Approximately 40% of the Italian apricot production comes from the Campania region, where the most important area of cultivation is around the Vesuvian area. Little is known about the genetic identity of the Vesuvian apricot trees. Although several biotypes have been described, only 11 cultivars are accepted for the production of certified fruits. These cultivars, particularly adapted to the Vesuvian environment, are the result of selection carried out by local farmers. Therefore, it is important to define tools to objectively identify each cultivar in order to guarantee their conservation and protection. The objective of the present study was to provide a molecular marker-based profile to identify and discriminate the apricot cultivars protected by the “Albicocca Vesuviana” PGI mark. With this aim, the molecular diversity of these cultivars was studied with 7 SSR (Simple Sequence Repeats) analyzing loci. The allelic profiles discriminated 8 out of the 11 cultivars, whereas the other cultivars represent possible cases of synonyms or genetically close varieties. A more detailed morphological characterization of these varieties will allow a better understanding of the genetic relationship among them.

INTRODUCTION

The apricot, Prunus armeniaca L. (2n=16), is a species of the Rosaceae family probably domesticated in western China and Central Asia. Although widely diffused throughout the world, the top apricot producers are countries around the Mediterranean basin and in the Middle East, with the notable exception of Japan. According to FAO, Italy is the third largest producer in the world, after Turkey and Iran. Approximately 40% of the Italian production comes from the Campania region, where the most important area for apricot cultivation is around Mount Vesuvius. Despite yearly fluctuations in yield, this area accounts for 55-60% of the entire regional production, and just under a fifth of the Italian production. A considerable amount of the fruits (up to 80%) is used for the production of juices, jams and pastes, while the remaining is almost exclusively sold in local markets.

One of the main reasons of the success of the apricot cultivation around Mount Vesuvius is related to the mineral properties of the volcanic soil that make the “Vesuvian apricot” a tasty fruit of great nutritional value, rich in potassium, provitamin A and antioxidant properties (Di Vaio et al., 2003). Unfortunately, the surface grown with the traditional apricot cultivation is progressively being reduced, mainly because of the urbanization of rural areas.

In order to protect the Vesuvian Apricot production, a Protected Geographic Indication (PGI) is shortly to be registered at the European Union. This trade name is crucial for different reasons, such as the promotion of a product with superior characteristics, the improvement of the income of farmers (in return for a "genuine effort to improve quality"), the preservation of rural areas around Mount Vesuvius, and to give accurate information about product origin that is easily identifiable by consumers.

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certified fruits. These cultivars are the result of an implicit selection of local farmers and are particularly adapted to the Vesuvian environment. Several DNA-based markers have been used to define the genetic variability of apricot such as RFLP (De Vicente et al., 1998), RAPD (Takeda et al., 1998), SCAR (Jun and Chung, 2008) and AFLP (Hurtado et al., 2002). Simple Sequence Repeats (SSR) are one of the most frequently used molecular markers for characterization of plant germplasm, as they are abundant in plant genomes, highly variable and suitable for high-throughput applications (Schlotterer, 2004). For instance, in the Rosaceae family, SSRs have been used for genetic characterization [e.g. in plums (Ahmad et al., 2004) and sweet cherries (Wunsch and Hormaza, 2002)], comparative mapping studies (Cipriani et al., 1999) and traceability in agro-food chain [“Melanzane Campana” PGI (Melchiade et al., 2007)].

The present study describes the molecular characterization by SSR markers of apricot varieties included in the “Albicocca Vesuviana” (Vesuvian Apricot) PGI regulation.

MATERIALS AND METHODS

Plant Material


DNA Extraction and Data Collection

Genomic DNA was isolated from young leaves using a CTAB-based protocol (Melchiade et al., 2007). Polymerase chain reaction (PCR) amplifications were carried out using an Eppendorf Mastercycle Gradient thermocycler. To genotype plants, seven primer pairs previously developed in apricot (UDAp407, UDAp410, UDAp411, UDAp420, UDAp480, UDAp446) (Messina et al., 2004) and in peach (UDP97-402) (Testolin et al., 2000) were used. The forward primers were fluorescently end-labelled for capillary electrophoresis detection. PCR reactions were carried out in a volume of 10 µl containing 10 ng DNA, 50 mM MgCl₂, 1 mM dNTP, 0.5 U Taq DNA polymerase (Invitrogen), and 10 pmol of each primer in 1 X reaction buffer (Invitrogen). Reactions were performed using the following cycling conditions: 5 min denaturation at 94°C, followed by 35 cycles of 30s denaturation at 94°C, 30s at appropriate annealing temperature and 1 min elongation at 72°C. The last cycle was followed by a final incubation for 5 min at 72°C. Verification of product amplification was performed by electrophoresis in 1% TBE buffer at 8 V/cm across 2% (w/v) agarose gels stained with ethidium bromide.

For capillary electrophoresis, fluorescently labelled PCR products were mixed with 0.5 µl of GeneScan-500 ROX size standard (Applied Biosystems) and 8.5 µl of Hi-Di Formamide (Applied Biosystems), and resolved on an ABI PRISM 3100 Avant (Applied Biosystems). Allelic sizes were calculated by GeneScan 3.7 software (Applied Biosystems).

Data Analysis

Cultivars showing a single amplified fragment were considered, by convention, as homozygous at that locus. Subsequently, GeneAIEx 6 (Genetic Analysis in Excel) software (Peakall and Smouse, 2006) was used to calculate allele frequencies, number of alleles observed (Na), number of expected alleles (Ne), informative index (I), observed heterozygosity (Ho), expected heterozygosity (He) and the fixation index (F). Dissimilarity between samples was calculated using the Simple Matching coefficient, \( d_{ij} = 1 - \frac{1}{L} \sum (m_{ij}/\pi) \), where \( d_{ij} \) is the dissimilarity between units i and j, L the number of loci, \( \pi \) the level of ploidy and \( m_{ij} \) is the number of matching alleles for locus l. Distances were used to construct a dendrogram using the Neighbor-Joining method. The reliability of the
inferred tree was tested by bootstrap technique (500 resamplings). Calculations were performed by DarWIN software.

RESULTS
Molecular fingerprinting was carried out using 7 previously described SSR primers (Testolin et al., 2000; Messina et al., 2004) on two trees of 11 Vesuvian cultivars. Polymorphisms were detected at all the loci. A total of twenty-seven different alleles were scored, with an average of 3.85 alleles per locus. The allele frequency per locus ranged from 67% to 4.2%, with an average of 26%. The number of effective alleles was relatively high (a mean of 2.92 alleles). Overall, the data indicated the presence of a good level of polymorphism in the analyzed population. The observed heterozygosity largely differed among loci, ranging from 0.18 for UDAp411 to 0.95 (UDAp410). The Fixation index of UDAp411 and UDAp 480 loci was substantially positive, indicating the possible presence of undetected null alleles. Samples of the same cultivar showed the same profile with two exceptions. The first refers to ‘Boccuccia Spinosa’, as the two trees presented one allelic difference. At the UDAp480 locus, one of the trees had a single peak (143 bp), while the second was heterozygous (143 and 181 bp). Differences among samples labelled with the same name were also found for the ‘Boccuccia Liscia’. One sample (Boccuccia Liscia 2) had the same allelic profile of the ‘Fracasso’ cultivar, and it is likely to be a case of incorrect denomination. Molecular analysis led to the identification of a specific allelic profile for ‘Boccuccia Liscia’, ‘Vitillo’, ‘Fracasso’, ‘Baracca’, ‘Boccuccia Spinosa’, ‘Monaco Bello’ and ‘Ceccona’. Conversely, the SSRs employed did not allow the discrimination between ‘Pellecchiella’ and ‘Portici’ and between ‘San Castrese’ and ‘Palummella’, although they differed from other cultivars.

The genetic relationships of the Vesuvian PGI cultivars were analyzed by a distance-based phylogenetic analysis and the NJ dendrogram is reported in Figure 1. Samples of the same cultivar that showed the same allelic profile at every locus are presented as varieties.

DISCUSSION
Several studies indicated SSRs among the most suitable DNA markers for cultivar identification and analysis of germplasm evolution, owing to their high polymorphism, reproducibility, ability to differentiate individuals, genome coverage, and the ease of application (Schlotterer, 2004). Furthermore, the possibility of using SSR primers developed in closely related species to detect inter-varietal variation allows overcoming the trouble of their development in apricot. To fingerprint the Vesuvian PGI apricots, we selected microsatellite that showed the highest information content. The molecular analysis indicated slightly lower values of detected alleles and observed heterozygosity than those reported by Messina et al. (2004) and Testolin et al. (1999), yet similar to the ones reported in other studies (Hormaza, 2002; Romero et al., 2003; Zhebentyayeva et al., 2003). Furthermore, the average fixation index of our samples, a measure of population differentiation based on genetic polymorphism data, showed a lower value when compared to the one reported by Romero et al. (2003) but similar to the one obtained analyzing Tunisian apricots (Khadari et al., 2006). Considering that the trees analysed in the present study are cultivated in a limited geographic area, the data indicated a significant polymorphism and the presence of a potentially interesting level of heterozygosity in the Vesuvian varieties. In order to estimate the genetic relationship among samples, we performed an NJ analysis upon the dissimilarity data matrix (Fig. 1). The tree allocated the cultivars in well separated branches. Differences among samples under the same denomination were found for two varieties, ‘Boccuccia Liscia’ and ‘Boccuccia Spinosa’. As for ‘Boccuccia Spinosa’, there was only one different allele at the locus UDAp480. Inter-varietal differences are present in vegetatively propagated plants, especially fruit trees (Rao et al., 2009). In our specific case, the difference could be due to the presence of a null allele. This hypothesis is consistent with the Ho and He values of the UDA p480 locus (Table 1). As for the ‘Boccuccia Liscia’ variety, the
The presence of a case of erroneous denomination is very likely, as the allelic profile of one ‘Boccuccia Liscia’ tree matches the ‘Fracasso’.

The genetic analysis indicated that ‘Portici’ and ‘Pellecchiella’, and ‘San Castrese’ and ‘Palummella’ could be considered as possible synonyms, because of the numerous morphological descriptors they share (Della Strada et al., 1989), and the very scarce information available on ‘San Castrese’. On the other hand, ‘Portici’ and ‘Pellecchiella’ were described as different varieties (Della Strada et al., 1989) and thus our data suggested that this cultivars should be genetically very closely related. A previous study demonstrated that five SSRs were sufficient to discriminate 54 apricot landraces (Krichen et al., 2006). Similar numbers of microsatellites are generally sufficient to discriminate a high number of tree varieties. For instance, 6 SSRs distinguished 106 olive genotypes (Sarri et al., 2006) and 3 SSRs discriminated 49 palm cultivars (Zehdi et al., 2004). Nonetheless, in the future, it will be interesting to extend the molecular analysis to other loci, or to use other highly informative markers for the identification of intra-varietal differences (Rao et al., 2009).

CONCLUSIONS

In conclusion, the results of the present study indicated the presence of a good genetic differentiation in tree population analyzed and confirmed that SSRs are a powerful tool to reveal the presence of some inconsistencies that should be further analyzed.

Literature Cited


Tables

Table 1. Genetic indexes of the Vesuvian Apricot PGI cultivars relative to the analyzed SSR loci. (Na: No. of different alleles; Ne: No. of effective alleles; I: Shannon's information index; Ho: Observed heterozygosity; He: Expected heterozygosity; UHe: Unbiased expected heterozygosity; F: Fixation index.)

<table>
<thead>
<tr>
<th>Locus</th>
<th>Na</th>
<th>Ne</th>
<th>I</th>
<th>Ho</th>
<th>He</th>
<th>UHe</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>UDAp407</td>
<td>4</td>
<td>2.77</td>
<td>1.18</td>
<td>0.91</td>
<td>0.64</td>
<td>0.65</td>
<td>-0.42</td>
</tr>
<tr>
<td>UDAp410</td>
<td>5</td>
<td>4.38</td>
<td>1.53</td>
<td>0.95</td>
<td>0.77</td>
<td>0.79</td>
<td>-0.24</td>
</tr>
<tr>
<td>UDAp411</td>
<td>3</td>
<td>2.66</td>
<td>1.04</td>
<td>0.18</td>
<td>0.62</td>
<td>0.64</td>
<td>0.71</td>
</tr>
<tr>
<td>UDAp420</td>
<td>4</td>
<td>3.05</td>
<td>1.23</td>
<td>0.73</td>
<td>0.67</td>
<td>0.69</td>
<td>-0.08</td>
</tr>
<tr>
<td>UDAp480</td>
<td>3</td>
<td>2.65</td>
<td>1.03</td>
<td>0.36</td>
<td>0.62</td>
<td>0.64</td>
<td>0.42</td>
</tr>
<tr>
<td>UDAp446</td>
<td>5</td>
<td>2.22</td>
<td>1.10</td>
<td>0.64</td>
<td>0.55</td>
<td>0.56</td>
<td>-0.16</td>
</tr>
<tr>
<td>UDP97-402</td>
<td>3</td>
<td>2.74</td>
<td>1.05</td>
<td>0.59</td>
<td>0.64</td>
<td>0.65</td>
<td>0.07</td>
</tr>
</tbody>
</table>
Fig. 1. Unrooted tree showing the relations of the apricot varieties. Numbers indicate bootstrap values, if over 60.