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Coffee species and varietal identification

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Abstract — There are serious economical reasons to pretend warranties in coffee species and varieties authenticity. Arabica adulteration with Robusta coffees, intentional or not, is carried out at different steps of the coffee chain, from plantation to beverage. We present a method based on a real-time PCR technique to perform: a) a *qualitative* analysis to evaluate the presence/absence of a species in a sample; b) a *quantitative* analysis to amplify Robusta samples only, making possible the detection of less than 5% of Robusta in a mixture. Arabica cultivars sold as specialty coffees can be identified and certified by fingerprinting using SSR markers.

Index Terms — adulteration, *Coffea arabica*, *Coffea canephora*, microsatellites, multiplex PCR, real time PCR, roasted coffee, traceability.

1 Introduction

Coffee is one of the most important products in the international market. The annual consumption exceeds 5 billions kilograms, wich corresponds to 500 billions cups. The genus *Coffea* contains more than 100 species, only two of which, *Coffea arabica* (known as Arabica coffee) and *C. canephora* (known as Robusta coffee) are commercially cultivated. Arabica produces high quality coffee compared to Robusta, and contributes with about 70% of the total world coffee production, being consequently sold at 2-3 times higher prices. Also, among Arabica coffee some cultivars are considered as specialty coffees with peculiar organoleptic characteristics and a very high commercial value. Thus, there are serious economical reasons to pretend warranties in the authenticity of coffee species and varieties. Arabica adulteration with Robusta coffees can be intentional or not and is carried out at different steps of the coffee chain, from plantation (one or both species can be cultivated by the same producer) to coffee beverage.

Green coffee authentication can be very useful for roasters, while that of roasted coffee (beans or ground) should be very interesting for retailers and consumers.

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The methods to distinguish Arabica from Robusta in coffee blends are presently based essentially on the chemical analysis of compounds such as sterols [1], chlorogenic acid and caffeine [2], fatty acids [3], tocopherol [4], etc, but these do not always give reliable results. Nowadays there is no totally reliable method to guarantee coffee authenticity; the only reference is the production chain of coffee, trusting on what the sellers declare through labels of already packed products, or tasting the drink.

Concerning Arabica varieties, it is not possible to distinguish them from the morphology of the seed or from plant phenotype and agronomy. The only method to assess product quality wich is available for dealers is to roast the seeds and taste the coffee beverage. Importers also cannot know whether the small testing sample effectively corresponds to the many hundreds of coffee bags received afterwards.

In recent years, food forensics requires DNA-based methods for molecular analysis. The aim of this approach is to guarantee authenticity of commercially important foods that can be contaminated accidentally or by fraud. Generally, molecular tecniques based on DNA analysis are more effective and reliable than those considering phenotipic characteristics. In particular molecular markers such microsatellites or SSR (simple sequence repeats) are the most suitable for their features: abundance in eucariotic genomes, high level of polymorphism, codominance, locus specificity, PCR detection and high results reproducibility. SSR are widely used in the characterisation of plant species such as rice [6], potato [7], and wheat [8]

Research on coffee in this field is still at the beginning, and only one method based on PCR-RFLP is available [5]. Here a Real-time PCR based method is described for blend coffee analysis.

2 Materials and Methods

2.1 Discerning Arabica and Robusta

Real time PCR can be used to analyse coffee blends for establishing the relative presence of Arabica and Robusta coffee. This method relies on DNA-based probes which are complementary to target sequences in a region internal to PCR primers. Each probe has a fluorescent reporter at one end and a quencher of fluorescence at the opposite end of the probe. The close proximity of the reporter to the quencher prevents detection of its fluorescence; breakdown of the probe by the 5' to 3' exonuclease activity of the Taq polymerase breaks the reporter-quencher proximity and thus allows unquenched emission of fluorescence, which can be detected after excitation with a laser. An increase in the product targeted by the reporter probe at each PCR cycle therefore causes a proportional increase in fluorescence due to the breakdown of the probe and release of the reporter.

2.2 Qualitative analysis of Arabica and Robusta blends

The method is based on the amplification of both Arabica and Robusta samples through the use of Real Time PCR technology. The identification of these two species is given by tree different probes, each one presenting a specific fluorochrome: one is an universal probe, that recognizes both Arabica and Robusta, index of amplification efficiency; a specific probe for *C. arabica*, that binds a sequence present only in Arabica but absent in Robusta; a specific probe for Robusta, that binds a piece of DNA present in *C. canephora* and absent in Arabica. This method allows to carry out qualitative analysis to evaluate the presence/absence of a species in a sample. Additionally, it can also amplify and detect relative quantities of the two species in coffee blends.

2.3 Quantitative analysis of Arabica and Robusta blends

The second method permits only Robusta species amplification also in Arabica/Robusta blends, through the use of Real Time PCR technology. The detection of the DNA products is given by an universal probe, that recognizes both species. In addition, to avoid undesired amplification of Arabica, a LNA (Locked Nucleic Acid) oligonucleotide clamp was added. This clamp can hybridize in a DNA region present only in Arabica but absent in Robusta, and does not permit primer annealing. This method inhibits the amplification of the most abundant species (usually Arabica) in favour of the less abundant one (Robusta) that can be present in case of fraudulent contamination. Using this method it is possible to give an estimation of the percentage of Robusta present in a mixture.

2.4 Arabica coffee fingerprinting

DNA was extracted from leaves and seeds of 320 different plants of *Coffea arabica* that constitute the Arabica collection of Laboratory of Genetics (University of Trieste, Italy).

Two multiplex PCR reactions were performed on each sample. The M1 reaction involves 9 couples of primers and allows to amplify at the same time 9 microsatellite loci. The second multiplex, PCR M2, contains 7 couples of primers and allows amplification of 7 loci microsatellites. Moreover, the amplification products of M1 and M2 primers are studied to have amplicons with non-overlapping molecular weights. Since this is not always possible, primers are labelled with different fluorophores to distinguish amplification products with similar size. This permits to mix both M1 and M2 products and to analyze them in a single electrophoretic run through the genetic analyzer. The sequences on which primers contained in M1 and M2 were designed are covered by patent [9]. The advantages of this technique are: PCR easy to perform; possibility to analyze 16 microsatellites using only 2 PCR reactions and one run by the genetic analyzer, saving reagents, time and costs.

3 Results and Discussion

3.1 Qualitative and quantitative analysis of Arabica and Robusta blends

Two methods were developed to distinguish Arabica and Robusta species. The qualitative approach permits immediate verification of the presence of Arabica, Robusta or of both in a mixture. Furthermore, we can approximately estimate the quantity of the two species up to 20 % of Robusta. Fig. 1A and Fig. 1B show the specificity of the two fluorescent probes for Robusta and Arabica coffee, respectively. The universal probe (indicated in Fig. 1A and 1B with number 1) gives an amplification efficiency higher than the other two (numbered with 2 and 3).

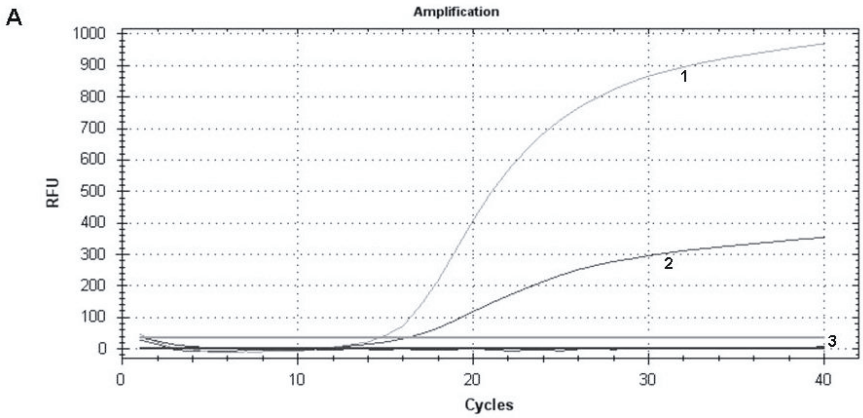

Fig. 1A – Amplification of a Robusta sample. The amplicon detection is given by the universal probe (1) and the *C. canephora* specific one (2), while the Arabica specific probe (3) shows no signal.

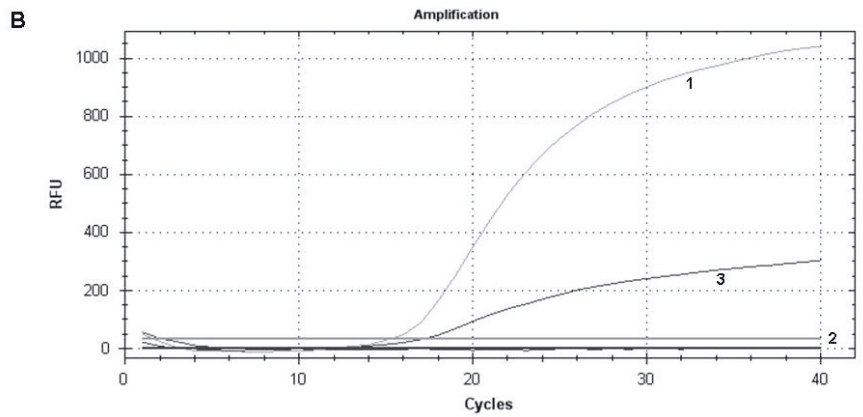

Fig. 1B – Amplification of an Arabica sample; the detection in this case is achieved by the universal probe (1) and the Arabica specific one (3), while the *C. canephora* specific probe (2) gives no signal.

The quantitative method was developed to amplify only Robusta samples, making possible the detection of less than 5% of Robusta in a mixture. The addition of the oligo clamp was required to inhibit Arabica amplification performed by this system. Fig. 2 shows that increasing concentrations of this oligo clamp progressively inhibit amplification of Arabica.

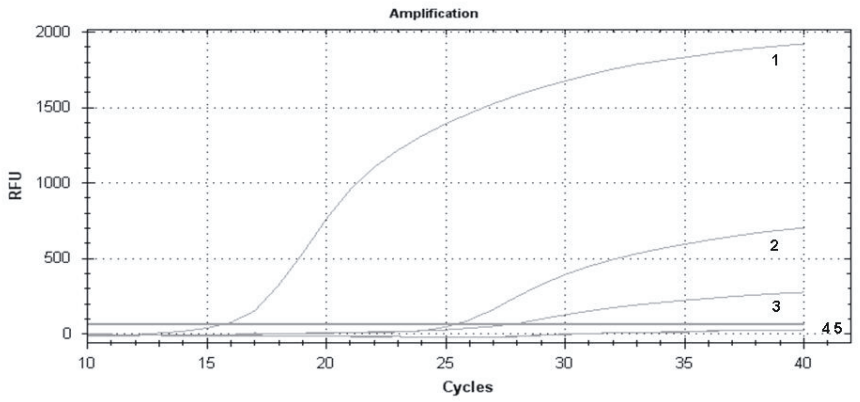

Fig. 2 – Chart showing amplification of Robusta and Arabica with the quantitative system. Curves 1 and 2 represent Robusta and Arabica amplifications, respectively. Curves numbered from 3 to 5 display progressive reduction of Arabica amplification with addition of increasing oligo clamp amounts, in particular: 0,06 µM (3), 0,6 µM (4), 1 µM (5).

Conversely, the oligo clamp doesn't interfere with Robusta amplification (data not shown).

This method can be useful to detect a wide range of Robusta percentages that can be present in a mixture.

3.2 Arabica coffee fingerprinting

Multiplex PCR reaction was performed successfully, giving a genetic profile for each of the 320 Arabica samples, as shown in Fig. 3. Alleles were used to calculate genetic distances and to design a cladogram showing genetic relationships between the samples. These varieties, whose origin is known over confidence, constitute a "genetic bank" that can be used to compare samples with unknown origin.

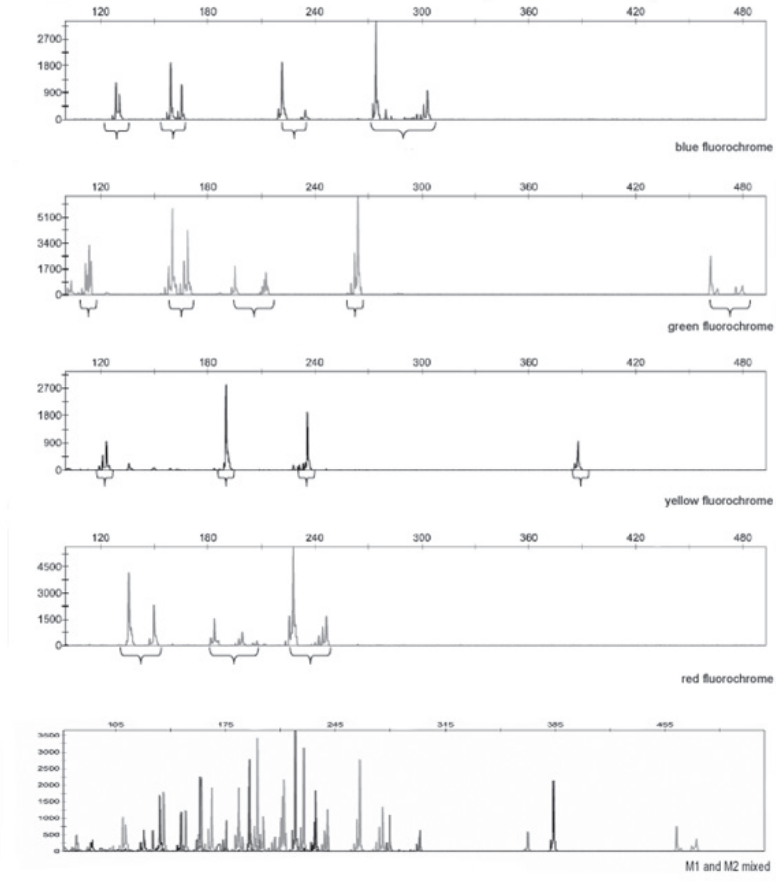

Fig. 3 – Example of sample amplification by Multiplex PCR.

4 Conclusions

All methods described in this paper can be successfully applied on green and roasted coffee beans with DNA extraction and analysis protocols set up in the Laboratory of Genetics of the University of Trieste. The main goals of these analyses aim towards food traceability. The authenticity of Arabica, Robusta or blends of the two species, is an important topic for producers and customers. Several molecular markers are available to establish the origin of coffee varieties for scientific aims, but none of them was used and validated for commercial purpose so far. The possible applications are many: analysis of a coffee stock proposed to a wholesaler, commercial coffee analysis to protect the dealer from unfair competition (food traceability). The analysis can be also used to determine the variety in a gourmet coffee lot.

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