Development of a genetic method for Olea europaea varietal identification

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Abstract

For several years, olive oil has been the subject of fraudulent scandals, mainly in the food industry but also in cosmetics. To identify this fraud, in recent years, genetic analyses have demonstrated their robust ability to both authenticate plant raw materials and trace them in processed products. The aim of this study is the development of new molecular tools for Olea europaea varietal identification. To respond to this aim, two microsatellite type molecular markers (SSR) were selected and tested on a data set comprising eighteen leaf olea europaea varieties reference samples. The results show that many reference varieties could be discriminated. These results allowed us to validate markers and method. The application of this method of olive oil will make it possible to address the problem of fraud and adulteration.

Number	Species	Sample type	Origine
Reference samples			
1	*Olea europaea subsp.* ***Europaea***	Leaf	France
2			France
3			France
4			Italy
5			France
6			Italy
7			Italy
8			Italy
9			France
10			Mediterranean region
11			France
12			France
13			France
14			France
15			France
16			France
17		Young leaf	France
18	*Olea europaea subsp.* ***Cuspidata***		South of Africa
Unkonwn samples			
Oil sample			
H1	*Olea europaea subsp.* ***Europaea***	Virgin olive oil	France

Table I: Presentation of samples

The table shows reference samples, unknown samples and oil sample, including species, types of sample (plant tissue) and geographical origins. The varieties was selected for the study from France and Italy are among the most widely used varieties for oil production.

Sample	SSR Markers		Combine result of two SSR markers
	OE-1	*OE-2*	
Olive Oil	Variety 1, 2, 7, 13, or 15	Variety 1, 2, 5, 6, 7 or 16	Variety 1, 2 and 7

Table 2 : Results of olive oil microsatellite analyses

The table 2 shows the microsatellite analysis results with OE-1 and OE-2 markers on olive oil and the final result with the combination of these two results.

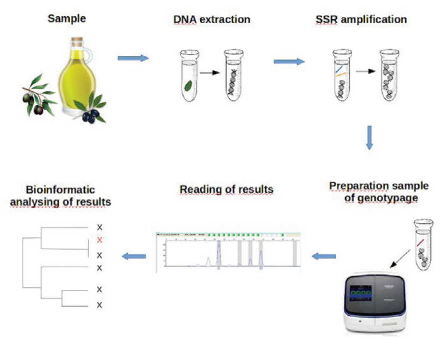

Figure 1: Steps of botanical identification with microsatellite genetic markers

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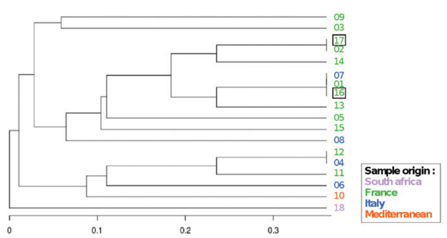

Figure 2: Phylogenetic tree of Olea europaea subsp. europaea samples

The phylogenetic tree is built by R with UPGMA with the combine results of two SSR markers. Unknown varieties are framed in black. Varieties written in green, blue, red and purple are respectively of French, Italian, Mediterranean and South African origin. Very different patterns are observed between southern European and South African varieties.

Introduction

The olive tree, Olea europaea L. (Oleaceae, 2n), is a characteristic tree of the Mediterranean basin. Among the six subspecies of Olea europaea, the subspecies europaea includes about two thousand varieties of olive trees as well as the wild variety, the oleaster (Olea europaea subsp. Europaea var. Sylvestris). They are distinguished by a diversity of morphological, phenological and chemical characteristics (Breton et al., 2006). Already coveted since antiquity, women used olive oil and fruit from the olive tree for body care. Today, the olive tree is used in many industrial sectors. In food, the oil extracted from olives as well as table olives are essential elements of the Mediterranean diet. Known for their moisturizing virtues and antioxidant properties, olives and olive oil, rich in polyphenols, are also major components of some cosmetic and body care products (Omar et al., 2010).

In recent years, olive oil has been heavily affected by fraud, caused by the scarcity of raw materials and the high economic value. It may be adulterated with lower quality varieties or other oilseed species. This type of adulteration is frequent and affects many products in several sectors requiring controls on raw materials and products (Health and Food Safety, European Commission, Annual Report, 2018).

In this context, to control and fight against fraud, genetic analyses are efficient. They allow us to carry out the authentication of raw materials as well as their traceability in products. These analyses use genetic markers, which are widely used in genetic diversity studies and also for the identification of plant species. For example, microsatellite markers (called Simple Sequence Repeats (SSRs)), defined by a repetition of specific genetic patterns are used to identifiy plant varieties in agronomic field (Cipriani et al., 2002). They are identified by a pair of single primers framing the repeating pattern. SSRs are recognized as good markers because of their specificity and use (Gomes et al., 2018; Powell et al., 1996).

The aim of this study is to set up a new method for identifying plant varieties in raw materials and products. It will be based on the use of microsatellite-type molecular markers and new experimental protocols.

Materiel & Methods

1) Plant material

The study is carried out on sixteen authenticated leaf samples of French, Italian and wild (Mediterranean) geographical origin (called reference samples), two leaf samples of unknown variety of French geographical origin and one sample of olive oil of unknown composition.

2) Experimental protocol

The experimental protocol is shown in Figure 1. The DNA from each leaf sample is extracted and assayed. Two SSR markers (Oe-1 and Oe-2) were selected from the literature (Sefc et al., 2000) and amplified for each DNA extract, and then the fragments were analysed with a Sanger sequencer.

Results

1) Data study of leaf samples

The results are illustrated by a phylogenetic tree using the statistical and graphical software R (Ihaka R and Gentleman R., 1996). The tree shows the distinction of different subspecies and varieties. Phylogenetic tree shows O. cuspidata (sample 18) subspecies is different of the other samples. We can see that many varieties are differentiated except between the sample 02 and 17 ; 01, 07 and 16 ; 04 and 12. The figure 2 also shows the distinction between the two unknown species (17 and 18). The unknown varieties are respectively assigned at variety 01 or 07 for sample 16 and variety 02 for for sample 17. In order to expand the database, refine the genetic profiles and distinguish the samples, it would be necessary to analyse more reference samples with a larger set of markers.

2) Data study from olive oil sample

The table 2 shows the olive oil results. Genetic profiles could be established by comparaison with the leaf sample genetic profiles with the two SSR markers. The combination of SSR marker results shows the oil sample is constituted of varieties 1, 2 or 7.

Discussion

1) Study of the data

The results allow us to validate the olive variety identification method. These first very encouraging results demonstrate that this method is efficient to authenticate an olive variety in raw materials.

However, to improve this method and characterize whole dataset, it would be necessary to identify and use other SSR markers. Thus, we will refine the genetic profiles and discriminate all studied varieties.

Futhermore, to identify many olive varieties and secured their identification, it would be interesting to enrich the database with other reference samples.

The application of these tools of oil sample shows the possibility to work on product sample. Indeed, DNA analysis gives elements of plant composition of the oil. It's possible to detect several olive varieties.

In conclusion we can say that this method could be used to address the problem of fraudulent oil.

2) Advantages of this technique

Currently, with the increase of fraud and adulteration, the authenticity and traceability of raw materials in processed products have become essential for the manufacturers and the consumers. To address this issue, DNA Gensee decided to show its ability to guarantee the quality of raw materials and their traceability on olive oil with DNA technologies. The development of new molecular tools increases the skills in the identification of plants at the subspecies level. We can meet a more precise demand for authentication of olive varieties from raw material until in the oil.

Conclusion

This study introduces the implementation of a new olive identification technique using microsatellite markers.

It is based on sixteen authenticated varieties of Olea europaea and the subspecies Cuspidata. The DNA analyses make it possible to discriminate eleven reference varieties among seventeen. The method has been validated by distinguishing the two unknown samples, which are genetically close to some of the varieties present in the collection.

Then, this method has been applied on oil sample. The presence of several varieties of olives has been demonstrated.

This study, which is part of the problem of fraud and adulteration of olive oils, makes it possible to guarantee the safety of raw materials and products and the transparency due to the consumers.

Acknowledgment

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