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## Advances in DNA typing in the agro-food supply chain



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### ABSTRACT

**Background:** DNA typing is increasingly being applied to assess the genetic origin and authenticity of products entering and exiting the food supply chain. The growing interest in DNA typing has arisen from an expanding array of contexts, such as the need to protect manufacturers, ensure compliance with food regulations, validate labels, fight misbranding, evaluate product ingredients and defend consumers' rights and freedom of choice.

**Scope and approach:** This review presents current practices and emerging technologies about the genetic traceability in the agro-food chain, providing an overview of the specificity and challenges related to the analysis of commercial products of plant origin. We also discuss unsolved needs and specific features of DNA testing in the agro-food supply chain. These include the biochemical and physical variability of the samples under investigation, the possible DNA degradation, and the necessity to distinguish among plant varieties and not only different species.

**Key findings and conclusions:** We acknowledge that a number of DNA typing systems have been successfully used, and the vast majority are based on the PCR technique. Advances in next-generation sequencing technologies are expected to greatly expand data range and the amount of information accessible to a DNA analysis. The evaluation and implementation of novel technologies and tools, along with concerted efforts to increase information sharing and to establish standard operating protocols, are main priorities of genetic typing in the agro-food chain.

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## 1. Introduction

Plant genetics and biotechnology have made an essential contribution to the development of agriculture and human society by generating new varieties and plant-derived products with improved or novel characteristics (Duvick, 2001; Moshelion & Altman, 2015). Recent molecular and genetic advances can further assist the agro-food industry, thanks to the development of new analytical tools. Among the available applications, learning about the genetic identity of food and feed ingredients and detecting the presence of contaminating organisms have become increasingly popular even among non-specialists. DNA-based techniques for the resolution of legal cases and for clinical diagnosis have been covered so often in mass media that it is sometimes difficult not to oversimplify the power and the meaning of genetic testing (Caulfield & McGuire, 2012).

This review provides an overview of the main concepts and topics of the DNA analysis as they apply to the agro-food sector. The

aim is to highlight how molecular tools are important to protect producers, ensure consumers' freedom of choice and verify label accuracy. We present the main DNA typing systems, and scientific issues related to the genetic traceability of plant-derived food and commodities. In addition, we discuss how genetic traceability is a vital asset to promote and protect names of quality agricultural products and foodstuffs in the whole agro-food chain. Finally, we explore future developments related to the translation of the power of high-throughput sequencing technologies to genetic traceability for food testing.

## 2. Background

Scientific and technological advance in molecular genetics and genomics have substantially accelerated diagnoses based on the analysis of nucleic acids (Jobling & Gill, 2004; Kayser & de Knijff, 2011). Since the first pioneering applications of the 1980s, DNA-based techniques have grown tremendously in number and variety and over time, have become more reliable, accurate and powerful (Kayser & de Knijff, 2011). Although not yet commonly used as in human diagnostics and forensics, genetic analyses are

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rapidly being applied in the agro-food industry (Houlton, 2009; Madesis, Ganopoulos, Sakaridis, Argiriou, & Tsaftaris, 2014; Mafra, Ferreira, & Oliveira, 2008; Primrose, Woolfe, & Rollinson, 2010) and in forensic botany (Coyle, 2004; Hall & Byrd, 2012). Currently, DNA analysis for the agro-food sector constitutes a predominant part of forensic plant genetics, that is, the molecular and genetic study of organisms of agricultural interest in relation to legal issues (Hall & Byrd, 2012; Nybom, Weising, & Rotter, 2014).

Food fraud refers to the intentional partial or complete replacement, addition, removal or omission of food or food ingredients. The term includes also erroneous labeling and misleading statements about a product. In all these instances, DNA testing is able to verify the presence of a particular plant species or variety from farm to fork. Moreover, DNA typing of food-borne pathogens has become an indispensable tool to study patterns, causes, and risk factors of disease but this subject will not be covered in this review.

### 3. Authenticity of food deriving from plant species or varieties

In the context of traceability in the agro-food chain, aspects related to so-called “food authenticity” are very important (Primrose et al., 2010). An “authentic” food is one that conforms to the description provided by the manufacturer or producer, in respect to the origin of ingredients, the story of the transformation process, the geographic region of origin and the identity of the species or varieties used.

The partial or complete replacement with food or ingredients considered inferior to the reference product is a recurring problem (Bush, 2002; Taylor, 2010; Teletchea, Maudet, & Hanni, 2005). Although there have been alarming cases, this type of fraud rarely poses serious health risks and it is seen mainly as an economic fraud (Spink & Moyer, 2011). In addition to deception of consumers and unfair competition for producers, such fraud causes confusion in the market, disaffection towards genuine products and price fluctuation. For these reasons, food producers and control agencies need new analytical methods capable of guaranteeing the authenticity of the food during all stages of the supply chain. This requirement is boosted by consumers' increasing awareness of food safety, geographical origins, and methods of cultivation and processing (Skuras & Vakrou, 2002).

The tools for food authentication differ, and include isotopic analysis, chromatography, enzymatic analysis, spectroscopy, immunological or chemometric methods, as well as DNA-based analysis (Reid, O'Donnell, & Downey, 2006; Sun, 2008). The last tool has an essential importance for the genetic authenticity because any statement regarding the presence of materials/species in food necessarily involves a genetic investigation (Madesis et al., 2014; Martins-Lopes, Gomes, Pereira, & Guedes-Pinto, 2013; Primrose et al., 2010). Frequently, processed foods do not have any morphological feature that may be useful for the taxonomic classification of the organism of origin (Teletchea et al., 2005). Industrial processing makes less effective analyses carried out on other biochemical components of the food (Woolfe & Primrose, 2004). Moreover, DNA analysis enables determining, also in a quantitative way, whether the food from one species or varieties was mixed with material from other cheaper species or varieties (Woolfe & Primrose, 2004).

DNA-based techniques have proven to be effective in the identification of the species for a wide range of meat and fish, especially because they allow the classification of commercial samples that lack diagnostic morphological characteristics (Dalvit, De Marchi, & Cassandro, 2007; Rasmussen & Morrissey, 2008). Species identification is also a concern for plant products (Alary, Buissonade,

Joudrier, & Gautier, 2007; Ortola-Vidal, Schnerr, Rojmyr, Lysholm, & Knight, 2007). However, the information required for the genetic analysis of plant-derived products often goes beyond the characterization of the species, requiring reference to specific varieties or populations in cultivation (Korir et al., 2013). Therefore, the correct identification of the plant variety in food requires often a deeper level of genetic investigation. For many plant species, the market price of a fruit (and more generally, of an edible product) depends largely on the crop variety (Korir et al., 2013). Fraudulent adulteration could take place by replacing the declared cultivar with another of lower commercial value, to present a product that would still have acceptable organoleptic properties. A number of examples are listed in Table 1. This phenomenon includes imitations specifically intended to stand as equivalent to the authentic ones. These surrogates are often biochemically similar to the materials they intend to replace, making their identification and quantification difficult. The genetic traceability in the agro-food sector is therefore essential to distinguish traditional varieties with specific characteristics of high quality and, consequently, to protect quality EU-labels, such as Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI). Consumers associate these labels with quality, uniqueness and protection of producers (Bonnet & Simioni, 2001; Palmieri, Bozza, & Giongo, 2009).

### 4. DNA profiling of plant-derived food and agro-products

Despite the fact that food matrices are extremely complex and variable in composition, it is possible to isolate the DNA of sufficient quality from a range of products. The DNA is much more resistant to industrial processing (for example, high temperature and extreme pH) than other molecules, such as RNA, proteins or secondary metabolites (Martinez et al., 2003; Woolfe & Primrose, 2004). On the other hand, the industrial transformation causes a series of chemical and physical alterations to DNA molecules (Gryson, 2010; Tian, Guan, Wang, Teng, & Wang, 2014). The chemical degradation (e.g., abasic sites, cross-linking, oxidation, etc.) and physical fragmentation represent the most common modifications (Bauer, Weller, Hammes, & Hertel, 2003; Gryson, 2010; Meyer, 1999).

There are several methods to analyze the residual DNA in food to uniquely identify the species or the variety used (Madesis et al., 2014; Martins-Lopes et al., 2013; Meyer, 1999; Primrose et al., 2010; Teletchea et al., 2005). In theory, any polymorphic DNA marker could be used. Among the commonly available techniques, the PCR-based methods have offered the most interesting contributions (Jobling & Gill, 2004), mainly because the PCR provides qualitative or quantitative information even when there is a reduced amount of template DNA. In addition to the fluorescent-based quantitative PCR approaches, the droplet digital PCR assay offers an increased accuracy with tiny amount of DNA targets and exhibits a good tolerance to inhibitors (Morisset, Stebih, Milavec, Gruden, & Zel, 2013). Moreover, the PCR enables the simultaneous amplification of multiple target molecules in the same reaction (multiplex PCR), thereby enabling an increase in the output/cost ratio (Archak, Lakshminarayanareddy, & Nagaraju, 2007; Arlorio et al., 2003; Consolandi et al., 2008). Table 1 reports some examples of the most common techniques used for DNA testing of plant-derived products. The standard PCR is arguably the less expensive technique for DNA analysis. It can be easily transferred to analytical laboratories that do not possess a specific experience because numerous reagents and commercial kits are commercially available and little equipment is required.

#### 4.1. DNA isolation

The PCR in the agro-food chain, regardless of the marker

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