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European validation of a Cannabis sativa 13-locus STR multiplex kit for genetic identification: A preliminary study

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ABSTRACT

Cannabis sativa L. is a plant cultivated worldwide as a source of fiber, medicine and intoxicant. Traditionally, is divided into two main types: fiber type (hemp) and drug type (marijuana). Marijuana differs from hemp by the presence of a high quantity of the psychoactive drug, Δ9-tetrahydrocannabinol. The development of a validated method using short tandem repeats (STRs) could serve as an intelligence tool to link cases by means of genetic individualization or association of cannabis samples. For this purpose, a 13-locus STR multiplex method was developed, optimized, and validated by the Department of Forensic Science at Sam Houston State University (SHSU) according to relevant ISFG and SWGDAM guidelines. The European community considers *C. sativa* plants illegals, even though its consumption is accepted in precise and limited places (coffee shops or cannabis clubs in Netherlands and Spain). However, there are different gaps in the legislation of some European countries. For instance, in Italy, "weed" possession is decriminalized. Although trafficking and sale are prohibited, possession of small quantities of marijuana is considered only a civil offense. In order to proceed with the kit evaluation and inter-laboratory comparison, SHSU DNA laboratory sent blind cannabis DNA samples of known genotypes. Blind DNA samples were analyzed in different laboratories with different sequencers and analysis conditions. In this article, the goals were: a) to demonstrate that 13-locus STR kit for *C. sativa* is robust enough and reproducible, in all forensic laboratories, and b) to show the applicability of the STR system in association with Cannabis sativa cases for intelligence purposes to link multiple cases by means of genetic individualization or association of cannabis samples.

1. Introduction

Nowadays, *Cannabis sativa*, even though it has been used and cultivated for at least 6000 years [4], remains one of the most discussed and controversial plants for the scientific community. Today, the focus of forensic analysis involving cannabis is within the toxicology area. Although the idea of analyzing cannabis DNA with genetic individualization purposes emerged in publications several years ago [5], with great evolution of techniques, genetic analysis has not yet been adopted as a routine.

Furthermore, the advantages the genetic individualization of cannabis plants could provide in the illegal trade investigations are enormous, since it offers the possibility of establishing relationships between different cases and assessing their links to a trade network [5].

2. Materials and methods

Four laboratories - Sam Houston State University (USA), Biogem Scarl (Italy), Università Magna Grecia Catanzaro (Italy) and Universitat de Barcelona (Spain) – participated in the inter-laboratory validation study of 13 STR kit multiplex for the identification of *Cannabis sativa*, developed by Houston et al. [1,2]. The multiplex consisted of cannabis STRs ANUCS501, ANUCS305, B05, CS1, D02,C11, H06, 9269, 4910, 5159, 9043, 1528, and 3735 [2,3]. Participants were provided with: 1) PCR primers and master mix - consisted of 6.25 µL of 2x HotStar Taq®Plus Master Mix (Qiagen), 1.25 µL 2 mM Primer-mix, 1.25 µL 5x Q solution (Qiagen), 0.4 µL 8 mg/mL bovine serum albumin (Sigma-Aldrich, St. Louis, MO), and 1.35 µL deionized H2O - for a 13-STR multiplex *Cannabis sativa* profiling assay; 2) an allelic ladder, consisting of

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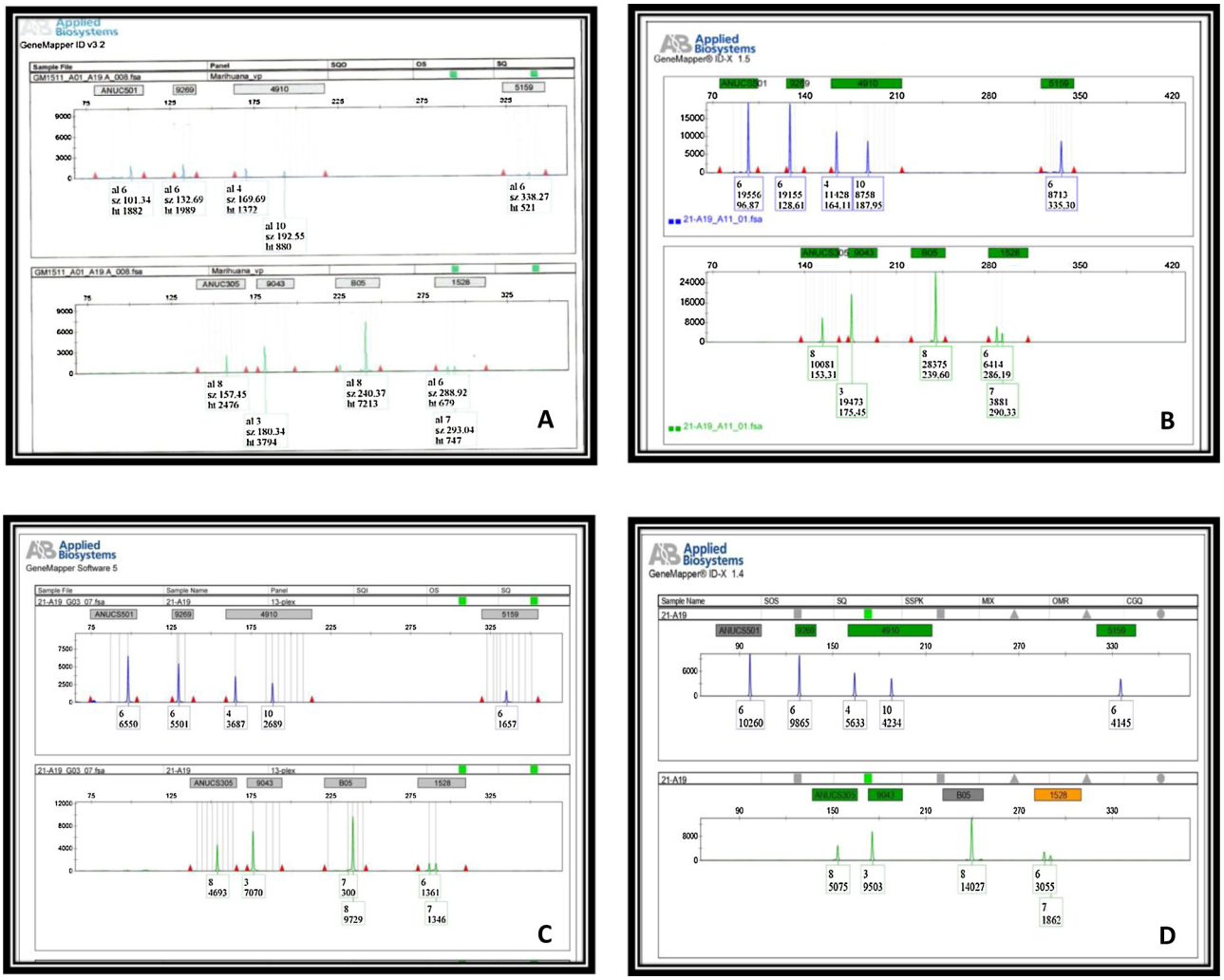

Fig. 1. Examples of partial electropherograms of loci analyzed by four forensic laboratories: ANUCS501, 9269, 4910, 5159, ANUCS305, 9043, B05 and 1528. Full concordance was observed among all laboratories: A) Universitat de Barcelona (Spain): B) Biogem Scarl (Italy); C) Sam Houston State University (USA); D) Università Magna Grecia Catanzaro (Italy).

56 alleles using the most common alleles observed for all markers [1,6]; 3) detailed protocols for PCR amplification, genotype, profiling and interpretation and 4) two blind samples. All laboratories were equipped with different instruments for forensic genetic analysis. Analytical and stochastic thresholds were set at 100 and 700 relative fluorescence units (RFUs), respectively.

3. Results

All laboratories were able to generate STR profiles and stochastic effects were not seen. All participants, despite using different platforms provided correct DNA profiling results. Hence, they showed full concordance for the blind samples. Samples of electropherogram comparison of 13-locus STR multiplex experiments are shown in Fig. 1.

4. Discussion

This validation highlighted the robustness and the applicability of *Cannabis sativa* 13-locus STR multiplex kit for genetic identification. Moreover, obtaining the correct DNA profile prpr with different instruments and parameters conditions supports the reproducibility, reliability, and accuracy of the method.

5. Conclusions

In summary, this European preliminary study is in line with the idea of a 13 loci cannabis STR multiplex can be used for forensic DNA profiling of *Cannabis sativa* samples in criminal investigations and international intelligence purposes. Moreover, data interpretation guidelines are being developed through an extended collaborative exercise, among several ISFG laboratories, prior to be routinely implemented in a forensic genetics laboratory.

Declaration of Competing Interest

None.

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