NUCLEAR, CHLOROPLAST, AND MITOCHONDRIAL DATA OF A US CANNABIS DNA DATABASE

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Since *Cannabis sativa* (marijuana) is a controlled substance in many parts of the world, the ability to track biogeographical origin of cannabis could provide law enforcement with investigative leads regarding its trade and distribution. Using autosomal, chloroplast, and mitochondrial DNA, we can not only predict the biogeographical origin of a plant, but we can discriminate between individual plants.

A previously validated 13-autosomal STR multiplex was used to genotype 496 samples. Samples were analyzed from four different sites: 21 seizures at the US-Mexico border, Northeastern Brazil, hemp seeds purchased in US, and the Araucarian area of Chile. In addition, we modified and optimized a previously reported multi-loci system to genotype five chloroplast and two mitochondrial markers. For this purpose, we designed a homopolymer STR pentaplex and a SNP triplex with one chloroplast (cscp001) marker shared by both methods for quality control. For successful mitochondrial and chloroplast typing, a novel real-time PCR quantitation method was developed and validated to accurately estimate the quantity of the cpDNA using a synthetic DNA standard. In addition, a sequenced allelic ladder was designed for the homopolymer STR pentaplex.

For autosomal typing, distinguishable profiles were generated from 381 samples that yielded full STR profiles and 44 duplicate genotypes within seizures were observed. Phylogenetic analysis and case-to-case pairwise comparisons of 21 seizures at the US-Mexico border, using *Fst* as genetic distance, revealed the genetic association of nine seizures that formed a reference population.

For mitochondrial and chloroplast typing, we performed subsampling and genotyped only 141 samples. Complete haplotypes (STRs and SNPs) were observed for 134 samples. As expected, extensive haplotype sharing was observed; five distinguishable haplotypes were detected. In the reference population, the same haplotype was observed 39 times with two unique haplotypes also detected. Haplotype sharing was observed between the US border seizures, Brazil, and Chile while the hemp samples generated a distinct haplotype.

Results revealed that both autosomal and organelle markers could discern population sub-structure. Phylogenetic analysis of the four populations using the neighbor joining method and *Fst* as genetic distance were estimated with the GDA software. Parsimony analysis was then performed with the *PAUP** software. And finally, the *STRUCTURE* software was employed to investigate the population structure among groups.