



B56 Investigation of the Genomics of Cannabinoid Biosynthesis in *Cannabis Sativa*

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After attending this presentation, attendees will: (1) be introduced to the biosynthetic pathway leading to the production of cannabinoids; (2) correlate the biosynthesis of cannabinoids with the underlying molecular characteristics of the genes encoding the enzymes that synthesize cannabinoids; and, (3) relate the molecular genetics of enzymes involved with cannabinoid biosynthesis with the potential to manipulate this species genetically to produce cannabinoids with medicinal properties.

This presentation will impact the forensic science community by contributing to a newer understanding of how variability at the DNA level in marijuana can significantly impact the chemical composition of this plant that holds promise as a source of natural medicines.

Currently, there are 23 states and the District of Columbia that have legalized the medical use of marijuana and its chemical products.¹ In addition, four states and the District of Columbia have legalized the recreational use of the plant as well.¹ The increasing legal use of marijuana and its products has stimulated research into the pharmacological benefits of the principal chemicals of interest produced by cannabis strains and also the biosynthesis of these compounds known collectively as cannabinoids. Two principal cannabinoids, delta-9-Tetrahydrocannabinol (THC) and Cannabidiol (CBD) have received the greatest attention to date; however, more than 60 cannabinoid compounds have been identified in cannabis.² Paralleling interest in the chemical composition of cannabis has been an interest in the genetics/genomics of the plant.³

The research discussed here has concentrated on two of the biosynthetic enzymes involved in the production of two of the principal cannabinoids with known medicinal effect, THC and CBD. The enzymes THC synthase and CBD synthase, respectively, are responsible for the conversion of Cannabigerolic (CBG) acid into the acidic forms of THC or CBD, which when heated, spontaneously become decarboxylated to the mature drug forms. The genes encoding THC synthase and CBD synthase are more than 80% homologous in sequence and exist in the genome as intronless genes of 1,635 basepairs.⁴

The THC and CBD synthase genes present in numerous seized cannabis samples were subjected to next generation sequencing on an Ion Torrent™ platform. Findings from these studies have indicated that the THC synthase and CBD synthase genes exist in cannabis in active and inactive forms based upon the Single Nucleotide Polymorphisms (SNPs) present within the coding sequence. In the case of the THC synthase gene, 30 SNPs have been detected within the 1,635bp coding sequence and behave as a “haplotype.” In other words, these SNPs travel together as a unit in different plants. It has been reported that some THC synthase genes are inactive as a result of SNP mutations that create stop codons or alter the amino acid sequence significantly. In hemp (which does not synthesize THC), this inactive haplotype is carried by both chromosomes, whereas plants producing THC genotype are either heterozygous for the inactive haplotype or homozygous for the active haplotype. Sequencing results showed that for CBD synthase, there were as many as 90 SNPs detected in the coding sequence for the gene. Future work involves obtaining additional plant materials rich in CBD to distinguish between the active and inactive haplotypes for the CBD synthase gene. It has been observed that seized marijuana may not exhibit appreciable amounts of CBD as would be expected from marijuana produced for illicit use even though it contained 3%-10% (by weight) THC.

Reference(s):

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***Cannabis Sativa*, Synthase Enzymes, Cannabinoids**