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Plant Cannabinoid Synthesis Evolution, Genetics, and Biochemistry: A Future Challenge for Biotechnology

Suzan Schil*

School of Biology and Environmental Science, University College Dublin, Ireland

Abstract

The psychoactive secondary compound tetrahydrocannabinol, or THC, is the most well-known feature of Cannabis sativa. THC, on the other hand, is just one of many phytocannabinoids found in this famous medicinal plant. The gradual legalisation of cannabis in many countries has created new opportunities for its medicinal and commercial applications, piqued scientific interest in the genetics and biochemistry of phytocannabinoid synthesis. Plant biology and genomics advancements help to accelerate research in the Cannabis field, which is still lagging behind other comparable high-value crops. We discuss the intriguing genetics and evolutionary history of phytocannabinoid synthases in this paper, as well as how a better understanding of Cannabis developmental genetics and morphology is critical for realising the full potential of phytocannabinoid production [1- 3].

Keywords: Plant genetics; Plant developmental biology; Flower development; Regulatory genes

Introduction

Cannabis sativa (Cannabis) has been described as the 'plant of the thousand and one molecules'. Phytocannabinoids, along with terpenes and flavonoids, are the most prominent molecules found in Cannabis. Tetrahydrocannabinol (THC) and cannabidiol (CBD) are just two of the more than a hundred phytocannabinoids that have been identified so far [4, 5]. The term phytocannabinoid refers to cannabinoids derived from plants, as opposed to cannabinoids produced by other organisms, such as the cannabinoids of the human endocannabinoid system. THC is a psychotropic substance that has been used for millennia for both recreational and spiritual purposes. However, it has become increasingly clear over the last two decades that THC and CBD, as well as other phytocannabinoids, have potential applications in the treatment of cancer and other diseases [6]. These discoveries have increased interest in studying the biochemistry of phytocannabinoid synthesis and its underlying genetics, the morphology and development of the associated plant structures, and the evolutionary origins of the synthases. This article summarises recent progress in those areas and discusses the implications for plant breeding and biotechnology [7].

Biochemical and genomic considerations in the complex world of phytocannabinoid synthases

In the plant, phytocannabinoid synthesis is a multistep process, including different precursor molecules which are further metabolised by four phytocannabinoid synthases: cannabigerolic acid synthase (CBGA synthase), tetrahydrocannabinolic acid synthase (THCA synthase), cannabidiolic acid synthase (CBDA synthase) and cannabicromenic acid synthase (CBCA synthase) [9]. CBGA synthase is a prenyltransferase, whereas THCA, CBDA, and CBCA synthases are all oxidocyclases that are closely related [8]. Oxidocyclase phytocannabinoid synthases are closely related to each other and belong to the family of BBE-like enzymes (a). Full-length CBCA synthase (CDCAS), THCA synthase (THCAS), and CBDA synthase (CBDAS) are closely related to uncharacterized pseudogenes and synthases, as indicated by clades A and B. An additional clade C has been identified, containing sequences that have not yet been thoroughly characterised [8].

Materials and Methods

Research area and sampling locations

A total of 81 Mansonia adult specimens were collected from eight different locations (RO-02 (n = 12), RO-03 (n = 10), RO-04 (n = 7), RO-07 (n = 13), RO-09 (n = 11), RO-11 (n = 10), RO-12 (n = 9) and RO-23 (n = 9). Specimens were collected along a 70-kilometer stretch of the Madeira River in the municipality of Porto Velho, Rondônia, Brazil. The collection area stretches from the Jaci-Parana district to about 20 kilometres west of the municipality of Porto Velho. The climate is classified as AW - rainy tropical, with average temperatures ranging from 21 to 34 °C and monthly rainfall ranging from 17 to 264 mm. The rainy season lasts from October to April, with transition periods in May and September.

Field collections were conducted in March 2020, near the end of the rainy season, when precipitation and river water levels were at their highest. A mosaic of fragments of primary tropical rain forest and agricultural areas surrounded the collection sites [9]. Two sampling methods were used: CDC light and barrier screen sampling. All specimens were morphologically identified to the species level identification key prior to DNA extraction. Only morphotype 1 (classified as Mansonia titillans) and morphotype 2 (assigned as "near" Mansonia titillans) were used in the study and are referred to as Mansonia spp. throughout the manuscript.

Sample preparation and sequencing

The heads and thoraces of mosquitos were separated from the rest of the body for DNA extraction using a sterile scalpel. Each specimen was extracted separately using a ReliaPrepTM Blood gDNA

***Corresponding author:** Suzan Schil, School of Biology and Environmental Science, University College Dublin, Ireland, E-mail: suzan.schil@ucd.ie

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kit (Promega, Madison, USA) per the manufacturer's instructions. Fluorometric quantitation of DNA was carried out using a Thermo Fisher Scientific QuBit dsDNA HS Assay Kit according to the manufacturer's instructions.

According to the manufacturer's recommendations, DNA libraries were prepared using one-fifth of the total recommended volume for the Nextera XT Library prep kit (Illumina). DNA was extracted from 81 samples, multiplexed, loaded onto two mid-output flowcells, and sequenced on the NextSeq500 platform [10].

Results

Variance analysis and genetic variation

Eight essential oil constituents were identified in both populations' families. Trans-anethole, fenchone, estragole, and limonene were the main constituents. The ANOVA results revealed that there were significant differences (P 0.05) in all traits between the normal and water deficit conditions in the S1 and OP populations. With the exception of -terpinene in the S1 population, the effect of family was significant for all measured traits, indicating significant variation among the selected families with a broad range for each trait. The interaction between family and environment was significant for seed yield per plant and essential oil yield in the S1 population, and for seed yield per plant, essential oil yield, -terpinene, and anisaldehyde in the OP one population [11].

Furthermore, the effects of year and family year interaction were non-significant in both populations for the majority of evaluated characteristics.

The genotypic coefficients of variation (GCV) for S1 and OP populations under normal and water-stress conditions are shown. Higher GCV values (GCV50%) were obtained in selfed progenies in normal conditions for -pinene, -myrcene, limonene, -terpinene, estragole, and anisaldehyde. Higher GCV values were observed for -pinene and estragole in water deficit conditions [12]. Under both moisture conditions, higher GCV values were observed in open-pollinated progenies for -pinene, -terpinene, estragole, and anisaldehyde. The genetic variation under water deficit was higher than normal for some traits, such as seed yield per plant, essential oil yield, and -pinene in the S1 population, and seed yield per plant, essential oil yield, -pinene, and -myrcene in the OP population. Under normal conditions, variation in the remaining traits of both populations was greater. Based on GCV, estragole, -terpinene, and limonene had a relatively higher range of genetic variation in the S1 population, while seed yield per plant, trans-anethole, fenchone, -pinene, and essential oil yield had a relatively lower range of genetic variation. -terpinene and anisaldehyde showed a greater range of genetic variation in the OP population, as did estragole, seed yield per plant, essential oil yield, limonene, and –myrcene had a relatively lower range [13].

Mean comparisons of traits

Mean comparisons of S1 and OP populations for essential oil components are provided. Trans-anethole ranged from 11.24% to 95.97% with an average of 80.11% in the S1 population under normal conditions, and from 8.48% to 92.17% with an average of 77.95% in water deficit conditions. Families S1-35 had the lowest value of this component under normal conditions, while families S1-13 and S1-4 had the highest. Under water deficit condition, S1-35 had the lowest value of this component, and family S1-4 had the highest value of it.

Discussion

The findings of this study represent a comprehensive microgeographic analysis of the genetic structure of field-collected Mansonia titillans and Mansonia near titillans. In general, the availability of lentic habitats increases in response to anthropogenic environmental changes, such as the formation of large hydroelectric reservoirs and other minor water collections spread across the floodplain. As a result, native mosquito populations may become unbalanced, resulting in intense interspecific competition for breeding sites and, ultimately, the thriving of specific species. In the case of Mansonia spp., the formation of a lentic system favours the establishment of aquatic macrophytes, creating an ideal environment for oviposition and the development of immature mosquito stages. Furthermore, because Mansonia spp. females are aggressive biters with both exophilic and endophilic behaviour, human activities and the presence of livestock and poultry farming may influence the dispersal dynamics of these mosquito populations [14].

Conclusions

This study found evidence of Mansonia spp. genetic structuring near the SAE reservoir in Porto Velho, Rondônia, Western Brazil. This appears to be the first study to assess the microgeographic genetic diversity and dispersion of field-collected Mansonia spp. using a low-density whole-mitogenome sequencing protocol. Furthermore, this protocol could be applied to any mosquito species that requires a lower cost of NGS library construction and a lower sequencing effort. This molecular tool may also be useful for elucidating vector populations on a micro geographical scale.

Acknowledgement

None

Conflict of Interest

None

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