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An Update and Overview of Biotechnological Methods for the Creation of Potent Anticancer Compounds Derived from Plants

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Abstract

The plant world is a rich source of bioactive substances, many of which have been utilized therapeutically to treat a variety of diseases since prehistory. These metabolites have gained recent attention for their ability to treat a variety of malignancies using multiple pathways. Some of these substances include alkaloids or glycosides, such as podophyllotoxin (PPT), anaryltetralin lignan, and others that have shown promise as anti-cancer medications. Alkaloids come in three main types: quinoline alkaloids, diterpenoid alkaloids, and indole alkaloids. In this study, numerous plant biotechnology techniques that are employed to increase the production of these anticancer compounds in various species are discussed. Numerous in vitro culture methods, including hairy roots and stimulation of suspension culture, are useful in this area. In this context, a variety of in vitro culture techniques are being employed to study the effects of plant growth regulators and elicitors on different explants, including stimulation of suspension culture and hairy roots.

Keywords: Phytocannabinoid; Food security; Molecular farming; Biofuel; Edible vaccine

Introduction

The 'plant of the thousand and one molecules' is what has been said of Cannabis sativa (often known as marijuana). Together with terpenes and flavonoids, phytocannabinoids are the most prevalent compounds discovered in cannabis [1]. Of the more than one hundred phytocannabinoids that have been described so far, tetrahydrocannabinol (THC) and cannabidiol (CBD) are just two [2]. The term "phytocannabinoid" is used to characterise cannabinoids that are created by plants and to set them apart from cannabinoids made by other creatures, such as the cannabinoids found in the human endocannabinoid system. THC is a psychoactive substance that has been used for thousands of years for both recreational and religious purposes.

But during the past 20 years, it has become increasingly evident that THC, CBD, and other phytocannabinoids may be used to treat malignancies as well as mental and neurological conditions. These results have enhanced the need for research into the genetics and biochemistry of phytocannabinoid synthesis, the morphology and growth of the associated plant structures, and the evolutionary history of the synthases. This essay reviews recent developments in such fields and explores implications for plant breeding [3].

Since the 1980s, basic and applied research has been greatly helped by the development of gene transfer technologies (trans-genesis), and some of its products have been on the market since the mid-1990s. Here, these methods are referred to as "classical." Sequence-specific nucleases like Zinc Finger Nuclease, TALENs (Transcription Activator-Like Effector Nucleases), CRISPR-Cas systems (Clustered Regularly Interspaced Short Palindromic Repeats), and Oligonucleotide-Directed Mutagenesis (ODM) technologies are some of the sequence-specific nucleases that can be used to edit genes in plants [4]. Recent publications provide overviews of the application of CRISPR-based gene editing in plants, along with its difficulties and future opportunities. A report by German scientific authorities and an essay by Purnhagen and Wesseler both mentioned a number of legal issues and their ramifications while analysing the legal status and gene editing's effects in the EU [5].

The trichome is crucial for the production of phytocannabinoids, so all eyes are on me

While phytocannabinoids are produced in leaves at low levels, female inflorescences have by far the highest concentration. In comparison to many other angiosperms, the female Cannabis flower is significantly smaller: it has no stamens at all and develops a gynoecium made up of two united carpels that each contain one ovule. The bract that protects the flower is covered in stalked glandular trichomes, which are the primary locations where phytocannabinoids are produced [6]. Small subtending leaves on the inflorescence known as sugar leaves are coated with trichomes and produce phytocannabinoids.

Cannabis glandular trichomes are multicellular structures made up of a number of disc-shaped cells that secrete terpenes and phytocannabinoids [7]. The disc cells' outer zone of the outer cell wall loosens and expands during secretion, creating the trichome "head," an extracellular cavity where the secondary metabolites are made and stored.

At the subcellular level, the locations of phytocannabinoid production have only recently been identified. It is believed that the plastid produces CBGA, which is then transferred to the extracellular cavity. Additionally exported to the extracellular cavity are the enzymes for CBDA and THCA synthesis, meaning that the production of CBDA and THCA happens outside of the cell. CBCA is probably also produced outside of cells.

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The development of novel phytocannabinoids and the history of phytocannabinoid synthases

For thorough structure-function research and for advancing our understanding of phytocannabinoid production, an understanding of evolutionary links can be essential. Therefore, it is particularly intriguing to think about how phytocannabinoid synthases came to be. Since Cannabis lupulus, the closest relative of this plant, does not synthesise phytocannabinoids, the enzymatic machinery most likely developed after the two species split some 25 million years ago. Since THCA, CBDA, and CBCA synthases are all quite similar to one another, they probably come from the same ancestor [8]. According to phylogenetic reconstructions, CBCA synthase and THCA are more closely linked to one another than they are to CBDA synthase. Therefore, the current theory for the development of phytocannabinoid synthases maintains that one ancestral gene duplicated and diversified to produce the ancestors of THCA/CBCA and CBDA synthase.

A broad family of berberine bridge enzyme-like genes (BBE-like genes) that are present in bacteria, fungi, and plants includes phytocannabinoid synthases. Alkaloid biosynthesis, alcohol oxidation, and phytocannabinoid synthesis are among the diverse processes that are catalysed by BBE-like enzymes using FAD as a cofactor. It is unclear precisely how an enzyme that may catalyse the synthesis of phytocannabinoids originated from a precursor enzyme. The uncharacterized action of several BBE-like enzymes, including many of those closely linked to the phytocannabinoid synthases, further complicates analyses in this area [9].

In conclusion, by screening plants for novel secondary chemicals, there is great potential to discover novel pharmacologically intriguing phytocannabinoids. Sequence data alone may also be used to estimate a putative phytocannabinoid synthesis activity once structure-function correlations of BBE-like enzymes are better understood. This would significantly speed up the search for new phytocannabinoids given the abundance of genomic data now accessible. Additionally, employing directed evolution and/or rational design to manipulate the genetic makeup of other BBE-like genes or even existing phytocannabinoid synthase genes may result in the creation of novel phytocannabinoids with medicinal potential [10]. The THCA synthase's crystal structure has been determined, which might help efforts in this regard even more.

Understanding cannabis development for optimised plant breeding and cultivation

Cannabis is no different from other crops in that development biology is essential to crop improvement. The basis for novel adaptations and yield boosts will be a thorough understanding of the genetics and physiology of cannabis plant architecture, inflorescence, and flower development.

Cannabis plants were chosen for indoor cultivation, development under artificial light, and a highly branching yet compact architecture due to the drug's illegal status [11]. Even after legalisation, indoor growing is still widely used. However, due to the higher energy needs for lighting and temperature control indoors, outdoor production may be advantageous in many regions from an economic and environmental standpoint. Tall, heavily branched plants that are adapted to the local photoperiod may be the most promising for outdoor growing. breeding to produce cultivars with such qualities Understanding the genetic control of plant design, blooming time, and plant height in Cannabis, as well as the developmental subtleties involved, would be helpful. Although there has been some advancement in those fields, we are still far from having a complete knowledge of the genetics driving Cannabis development.

It should be remembered that various cultivars may react to environmental variations in different ways. It is obvious that further research is required in this area, although carefully selected conditions may very well be a key element in maximising phytocannabinoid output [12].

Heterologous expression systems and cell cultures

The ability to produce cannabinoids in cell cultures or other heterologous hosts has the potential to be very advantageous since it would allow for the creation and subsequent purification of specific cannabinoids while avoiding the legal issues related to cannabis growth. The toxicity of phytocannabinoids, which apparently precludes a high accumulation of the chemicals in cells, is one of the key challenges in developing heterologous expression methods. In extracellular compartments created by glandular trichomes (in Cannabis) and glandular scales (in Rhododendron), both plants manufacture and store phytocannabinoids [13]. Therefore, in addition to using a variety of enzymatic processes, the heterologous manufacture of cannabinoids in high concentrations may also need to develop a replacement for the highly specialised morphological structures that house phytocannabinoids.

Recent research shows that the yeast Saccharomyces cerevisiae is capable of producing cannabinoids. It's interesting that feeding cells precursors that are often not metabolised in the phytocannabinoid synthesis pathway resulted in the generation of novel cannabinoids, indicating the potential of this method for creating new cannabinoids. This and other heterologous methods still produce low yields, and various methods are being researched to overcome those restrictions, such as secreting cannabinoids in the culture medium where they can be readily removed and purified or producing cannabinoids in chloroplasts where they may be stored in higher concentrations. Although none of those technologies, to our knowledge, has yet matured to an industry-level, they continue to be promising means of manufacturing very pure and uncommon cannabinoids effectively.

Conclusion

Although it has been done in unconventional ways, breeding for Cannabis plants with higher THC levels or other phytocannabinoid profiles has a lengthy history. An rise in inflorescence density, trichome density, and increased activity of phytocannabinoid biosynthesis, among other things, have likely contributed to an increase in THC levels to as much as 20% of the dried flower mass. Cannabis research is currently expanding at never-before-seen rates and is supported by the giants of plant biology and crop science. It will only be a matter of time before methods like gene editing and plant transformation reach the same level of proficiency as other crops.

In order to produce plants with desired phytocannabinoid profiles, from hemp with no THC to novel phytocannabinoids, modern biotechnology and genomics will help to optimise phytocannabinoid synthases. Cannabis architecture, sex expression, and glandular trichome density and position will be improved with the aid of databases that capture natural genetic variability as well as marker-assisted breeding and gene editing.

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Conflict of Interest

None

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