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CHAPTER 7

RECENT DEVELOPMENTS IN OMIC-BASED APPROACHES IN HEMP (*Cannabis sativa* L.)

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INTRODUCTION

Dioecious hemp (*Cannabis sativa*), family Cannabaceae, grows as annual (Moliterni et al., 2004), plant species with gene center in Central Asia (Zhang et al., 2018). The seeds are a rich protein and vegetable oil (Yang et al., 2017) and the plants are rich fiber used in the manufacture of thread, clothes, paper money etc. (van der Werf, 1994). Due to the therapeutic properties of the high resin content; it has found use in medical, religious, cultural and recreational applications (Andre et al., 2016).

1. CANNABIS (*Cannabis* sp.); CLASSIFICATION AND IMPORTANCE

Some variations of the plants have narcotic effects and were prohibited for cultivation during the first quarter 20th century and is heavily regulated in several countries under the United Nations convention on narcotic drugs. (Bridgeman, 2017).

Its cultivation as medicinal plant has increased rapidly in recent years, scientific research has also increased in parallel (Dolgan, 2018) at the heart of this increase is the relaxation of legal limitations for its cultivation in some developed countries. With the controlled legalization of *Cannabis* and hemp-based products, a production and marketing network has emerged above the expected, reaching a market volume of billions of dollars in the USA alone (Parker et al., 2019; Conneely et al., 2021).

There are two basic compounds of Cannabis named THC (Tetrahydrocannabinol) and CBD (Cannabidiol). While cannabidiols

are mostly used for medical (sedative, anxiety prevention and sleep disorders) and cosmetic (shampoo, soap, skin care products) purposes, varieties containing high levels of THC are abused as drugs, although they contain more psychoactive components. Since 2001 (EC 2860/2000), the European Union has permitted the growth of fiber hemp types with a tetrahydrocannabinol (THC) level of 0.2 percent or lower in dry material. Varieties/species containing up to 5-20% THC mostly in dry material are called drug-type and are restricted due to their narcotic effects (Bruci et al., 2012). Cannabinoids are produced by phenols preserved in vacuoles and terpenes emitted by plastids in disc cells, according to the current hypothesis. Terpenes were discovered to be secreted from spherical plastids using electron microscopes.

2. OMICS-BASED APPROACHES

In its simplest definition, the suffix -omics functions as a derivational suffix (inspiration, neologism) in studies in the field of biology in English. Examples of omics are genomics, proteomics, and metabolics. Apart from these, transcriptomics and ionomics constitute a separate field of study in the field they specialize in. The field of omics research focuses on the collective characterization of an organism's molecules that translate into multiple biological structures, functions, and dynamics.

Omic-based methods; These uses high-throughput technologies such as proteomics, transcriptomics, ionomics, and metabolomics for large-scale analysis of various types of molecules. These high-throughput techniques are used on a large scale for purifying,

identification, and characterization of DNA proteins, RNA, and other compounds (Coughlin, 2014; Kumar et al., 2019).

Development of these methodologies have helped in rapid analysis of the compounds present in medicinal plants through integrated omic approaches (next-generation sequencing (NGS) technologies) methods (precursor feeding, overexpression, mutant selection). Analyses of huge and several samples could be performed rapidly, accurately and clearly in a short time (Porter and Hajibabaei, 2018). In addition, with the identification and characterization developed within the scope of these methods, the modification of the genes responsible for secondary metabolites can be included in homogeneous and heterogeneous systems. With the advancement of transcriptomics and genomics methods, it is now possible to describe several genes at the same time.

2.1. Genomics

The single gene level has been the main focus of molecular investigation of plants up until recently. This paradigm has been altered by recent technical developments, which now make it possible to evaluate organisms in terms of how their genomes are organized, expressed and interact.

Tremendous studies and data have been collected using genomic data since the beginning of the twenty-first century; more and more plants are being sequenced through for RNA and whole genome sequencing for characterization of genes to elucidate and regulate several metabolic and cellular pathways (Michael and Cristobal, 2013).

Genomic science focuses on genetics, material which are organized inside genome, using techniques used to gather and examine this data, and how this arrangement affects how the genes work biologically. As they rely on information recorded in DNA, genomic techniques permeate every element of plant biology and with expanded molecular analyses using a single level to multiple species. The previous paradigm to identify the genes underlying biological functions has been reversed by plant genomics, which now focuses on figuring out the biological functions underlying the genes. Additionally, it narrows the genetic gap between genotypes and phenotypes aiding in understanding of how a gene's genetic context and the genetic networks it interacts to modulate their activities in addition to the gene's isolated effects. (Campos-De Quiroz, 2002).

2.2. Transcriptomics

Transcriptome have important function in identifying potential genes involved in important metabolite biosynthesis for plants. Transcriptomics technology has gained momentum with next-generation sequencing (NGS) methods. Large-scale transcriptomes help define the mechanisms, cellular processes and responses of specialized metabolites in which candidate genes participate (Strickler et al., 2012).

2.3. Metabolomics

According to Hall et al. (2002), the term "metabolomics" refers to extensive analyses of complex metabolite mixtures in plant samples.

These simultaneous analyses facilitate complex metabolites in organisms and cells. It effectively analyzes the various chemical compounds induced in plant cells in this way (Pichersky and Gang, 2000).

2.4. Proteomics

Proteomic approaches include large-scale proteome analysis of tissues, organs and cells. It is an essential component of omics technologies and aids in the discovery of modifications in the proteome profiles of plants' responses to exterior factors. Proteome analytical tools for proteomic analysis are electrophoresis combined with the use of tandem mass spectrometry and electrophoresis have facilitated profiling in systematics one-dimensional polyacrylamide gel electrophoresis and two-dimensional electrophoresis combined with tandem mass spectrometry have enabled profiling of entire proteomes. (Hussain and Huygens, 2012). Proteomics approaches are useful in the identification of large-scale specific protein changes in organs, tissues and cells in response to the formation of plant-based formulations or purified plant-derived compounds. Their understanding the localization of proteins in various tissues may reveal information related to biosynthesis of specific metabolites (Martinez-Esteso et al., 2015).

3. RESULTS

Recently, omic-based methods have come to the fore in studies on plants with medicinal properties, including *Cannabis* (Aliferis and Bernard-Perron, 2020; Backer et al., 2020; Romero et al., 2020;

Vujanovic et al., 2020). When the omic-based studies on *Cannabis* are examined, it is seen that they mostly focus on trichomes (Aliferis and Bernard-Perron, 2020; Romero et al. 2020; Conneely et al., 2021). In a study by Andre and Guerriero (2016), *Cannabis* trichomes were named as small phytochemical factories. Trichomes, which are epidermal outgrowths, are primarily found on the Cannabis plant's leaf, bracket, and stem surfaces. The bracket and floral leaves of female plants are where glandular trichomes are most frequently found. Cannabinoids are therefore more abundant, in the tips of female plants. (Huchelmann et al., 2017; Monthony et al., 2021). As a defense mechanism, glandular trichomes have a function of secreting and storing secondary metabolites. Single or non-glandular trichomes are mentioned as non-glandular trichomes without any function as secretory organs. According to theory, non-glandular trichomes have genes that stop the plant from losing water or enable the synthesis of a few secondary metabolites. (McDowell et al., 2010; Monthony et al., 2021).

Because of their rich biochemical potential, some studies have characterized on the characterization of specialized trichome structures using omic methods. (Schilmiller et al., 2010; Jin et al., 2014; Aliferis and Bernard-Perron, 2020).

TrichOME (http://www.planttrichome.org/trichomedb/), an omics database on this subject, enables comparative analysis in plant trichomes and provides researchers with data mining about metabolites, genes and gene expressions (Dai et al., 2009). TrichOME is a functional omic database that includes mass spectrometry (MS)-based trichome metabolite profiles of both trichome and control tissues. A study

conducted by Conneely et al. (2021) can be used as an example for trichome characterization in this context. Researchers isolated the protein from the head and stem parts of the glandular trichomes (100 mg for each sample) as well as in flower samples (1 g) taken at the end of the flowering period. Protein samples were analyzed by nanoHPLC mass spectrometry method and protein identifications were compared with nanoHPLC MS/MS results with proteins from peptides from *C. sativa* cs10 Project (https://www.ncbi.nlm.nih.gov/genome/gdv/browser/genome/?id=GCF_900626175.1) with the reference proteome. Among a total of 1820 proteins, 370 (20%) were seen in all three samples; 569 proteins were detected only in flowers (31.3%), 122 proteins (6.8%) in glandular trichome heads and 1 protein (0.1%) in glandular trichome stems. In line with the results obtained; A total of 1682 proteins were found in the flowers of *C. sativa* taken at the end of the flowering period, 1240 proteins in the head of the glandular trichomes and 396 proteins in the stem of the glandular trichomes. The mitotic DNA binding, molecular functions and cell cytoskeleton, nucleozone, and microtubules were found to be overrepresented in the mitotoic cell cycle, molecular function, DNA binding, cytoskeleton, nucleozone, and microtubules as a result of Gene Ontology (GO) enrichment analysis. It has been determined that 569 proteins in flower samples are mainly effective in the biosynthesis of chlorophyll and in the photosynthesis process (Biological process and gene ontology: BP, GO terms). has been done. Therefore, many protein mapping studies have determined that glandular trichome head structures play a role in

terpene, phenylpropanoid, and phenolic biosynthesis tasks and are the center of secondary metabolites.

In addition to secondary metabolite synthesis, glandular trichome head structures were discovered. Glandular trichome stems, on the other hand, do not participate in biosynthesis and instead serve a variety of specialized functions on the trichomes. As a result, the main cannabinoids stored in the trichome heads contain at least 15% of the amount contained in the dried inflorescence of *C. sativa*. At the same time, trichome heads are important in the pollination period as they are a rich carbon source (Conneely et al., 2021).

Another omic-based study on *Cannabis* is carried out by Marti et al. (2014). Researchers subjected *C. sativa* leaves exposed to UV-C radiation, which has a metabolic inducing effect on secondary metabolite production, to LC-MS-based metabolomics and antioxidant analysis. There was no significant change in cannabinoid content as a result of the study, but dehydrostilbenes and cinnamic acid amide excessive derivatives. It has been reported that induced cinnamic acid amide derivatives have a functioning tissue damage cell wall strengthening. (Clarke, 1982). In the antioxidant activity results, however, no significant changes were observed in *C. sativa* leaves after UV-C radiation exposure.

Another metabolomic study on *Cannabis* is Pec et al. (2010) conducted by Researchers used jasmonic acid and pectin as elicitors. Jasmonic acid and pectin were used to treat cannabis cell cultures, and nuclear magnetic resonance was treated to the dual cell line. Chloroform extracts of pectin-treated cells differed from control and

jasmonic acid treatments, as shown by principal component analysis. In the methanol/water extracts, the cells treated with jasmonic acid showed a clear difference compared to the pectin and control treatments. Antioxidant metabolite tyrosol was found in *Cannabis* cell cultures, but it was more abundant when jasmonate acid elicitor was present.

4. CONCLUSION

A collection of newer and high-throughput methods, omics (genomics, metabolomics, ionomics, and metabolic engineering) are useful tools for large-scale production, diagnosis, and characterization of plant-derived substances in both in vivo and in vitro settings. Thanks to these new methods used, gene-protein-metabolite networks offer better opportunities in terms of increasing the production of specialized metabolites and molecular improvement. It is thought that understanding genetic, protein and metabolic activities and determining their mechanisms of action will be of great importance in plant breeding and secondary metabolite production in the future.

Omic methods used in hemp (*C. sativa*) plant were applied in drug types rather than fiber types. When omic-based studies in other medicinal plants are examined, it helps to better understand the protein-metabolism mechanisms of proteomic and metabolomic methods within secondary metabolites.

The controlled legalization of *Cannabis* plants and Cannabis-derived products in certain regions of countries such as the United States and Canada, particularly in the last 20 years, has created a very

large market volume and increased interest in these products. When examined on a product basis, secondary metabolites stand out mostly compared to fiber production, and technological developments (cannabinomics, proteomics, metabolomics) in this direction are increasing year by year. Since omics-based studies on *Cannabis* plants are a new field of study, the databases used are not plant-specific and bio-informatic analyzes of the data obtained include many databases. It is expected that in the coming years, with the concurrent formation metabolites and cannabis specific softwares, it will significantly contribute to Cannabis-related metabolomics applications.

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