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The overview of existing knowledge on medical cannabis plants growing

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Abstract: The use of cannabis for medicinal purposes dates back well before the era of modern medicine, but in recent years research into the use of medical cannabis in the medical and pharmaceutical sciences has grown significantly. In European countries, most cannabis plants have been and still are grown for industrial purposes. For this reason, hemp cultivation technology is relatively well researched, while little is known about the key factors affecting cannabis cultivation for medical purposes. The active substances of cannabis plant targeted by this review are called phytocannabinoids. The biosynthesis of phytocannabinoids is relatively well understood, but the specific environmental factors that influence the type and number of phytocannabinoids have been much less studied. Indoor or greenhouse cultivation, which uses automated lighting, ventilation, irrigation systems and complex plant nutrition has become much more sophisticated and appears to be the most effective method for producing medical cannabis. There are many different cultivation systems for cannabis plants, but one of the essential elements of the process is an optimal plant nutrition and selection of fertilisers to achieve it. This review summarises the existing knowledge about phytocannabinoid biosynthesis and the conditions suitable for growing plants as sources of medical cannabis. This review also attempts to delineate how nutrient type and bioavailability influences the synthesis and accumulation of specific phytocannabinoids based on contemporary knowledge of the topic.

Keywords: *Cannabis sativa* L.; tetrahydrocannabinol; cannabidiol; chemical profile; growing conditions

Cannabis is one of the earliest of domesticated crops. According to Chinese historical records and archaeological findings, its cultivation and utilisation can be traced back to 3 000 to 4 000 years BCE (Yu 1987, Jiang et al. 2006). The first use of cannabis for therapeutic purposes, directly evidenced by the finding of the stable cannabis compound, Δ^6-tetrahydrocannabinol (Δ^6-THC), has been dated to around 400 CE in a carbonised material discovered in a tomb at Beit Shemesh near Jerusalem (Zlas et al. 1993). Recent years have seen a boom in research on medical cannabis in the biomedical and pharmaceutical sectors. The applicability and acceptability of medical cannabis is expanding, as seen by the growing number of countries that allow its use for specific therapeutic indications (Shelef et al. 2011, Troutt and Didonato 2015, Balneaves and Alraja 2019). The number of active phytocannabinoids under investigation continues to increase and their effects on a variety of diseases such as chronic pain (Lynch and Campbell 2011, Portenoy et al. 2012, Wilsey et al. 2013), nausea and vomiting (Lane et al. 1991, Duran et al. 2010), spasticity (Pooyania et al. 2010, Corey-Bloom et al. 2012), depression (Wade et al. 2004, Selvarajah et al. 2010, Portenoy et al. 2012), glaucoma (Järvinen et al. 2002), inflammatory bowel disease (Ravikoff Allegretti et al. 2013), psychosis, motor and non-motor symptoms of Parkinson disease (Lotan et al. 2014), anxiety and sleep disorder (Russo et al. 2007, Bonn-Miller et al. 2014, Babson et al. 2017) are being studied (Doyle and Spence 1995, Järvinen et al. 2002, Lynch and Campbell 2011, Grotenhermen and

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Muller-Vahl 2012, Ravikoff Allegretti et al. 2013, Lotan et al. 2014). Nearly 150 different phytocannabinoid compounds are currently known (Hanuš et al. 2016).

TAXONOMY

History

The genetic plasticity of cannabis makes it difficult to catalog, and there is still a debate about its proper botanical classification. Linnaeus (1753) described *Cannabis sativa* as a single species. Based on comparative analyses of the psychoactive effects, leaf size, shape and structure of Indian and European varieties, Jean-Baptiste Lamarck (1786) classified the Indian cultivars as a separate species, *Cannabis indica*. At the beginning of the 20th century, the Russian botanist Janischevsky (1924) found that the local Russian plants possessed different characteristics from both *C. sativa* and *C. indica* yet still belonged to the cannabis taxon. These small, wild-growing, auto-flowering plants have been classified as a separate species named *Cannabis ruderalis* (Figure 1).

Current nomenclature

Small and Cronquist (1976) utilised a biphasic approach combining morphological and chemical characteristics to divide the *Cannabis* genus into the following four groups:

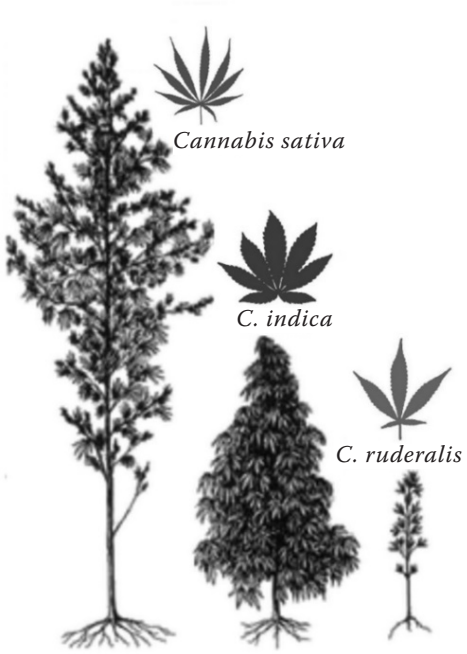

Figure 1. Species of cannabis (Hartsel et al. 2016)

1. *Cannabis sativa* L. subsp. *sativa* var. *sativa*,
2. *Cannabis sativa* L. subsp. *sativa* var. *spontanea* Vavilov,
3. *Cannabis sativa* L. subsp. *indica* Small & Cronquist var. *indica* (Lam) Wehmer,
4. *Cannabis sativa* L. subsp. *indica* Small & Cronquist var. *kafiristanica* (Vavilov) Small & Cronquist (Figure 2).

Hillig (2005) concluded from his genomic study of the classification of *C. sativa* that none of the previous taxonomic concepts sufficiently defined the *sativa* and *indica* genes. He analysed different genotypes from various geographical origins and was therefore inclined to a multi-species classification including *C. sativa*, *C. indica* and *C. ruderalis*. Small (2015) has recently proposed two possible cannabis taxonomic classifications. The first is consistent with an earlier division (Small and Cronquist 1976) and is in accordance with the International Code of Nomenclature for Algae, Fungi, and Plants (McNeill et al. 2012). The second, for domesticated cannabis, follows the guidelines of the International Code of Nomenclature for Cultivated Plants (Brickell et al. 2009):

Non-narcotic plants, domesticated for stem fiber and/or oilseeds in West Asia and Europe. Low Δ^9-tetrahydrocannabinol (THC) content and high cannabidiol (CBD) content (Hillig and Mahlberg (2004) *Cannabis sativa* "hemp biotype").

Non-narcotic plants, domesticated for stem fiber and/or oilseeds in East Asia, mainly China. From low to moderate THC content and high CBD content (Hillig and Mahlberg (2004) *Cannabis indica* "hemp biotype").

Psychoactive plants, domesticated in Southern and Central Asia. High THC content and low or absent CBD content (Hillig and Mahlberg (2004) *Cannabis indica* "narrow-leaflet drug (NLD) biotype").

Psychoactive plants, domesticated in Southern Asia (Afghanistan and neighboring countries). From moderate to high THC and CBD content (Hillig and Mahlberg (2004) *Cannabis indica* "wide-leaflet drug (WLD) biotype").

In addition, two hybrid classes have also been generated:

5. Non-narcotic plants, hybrid cultivars between two fiber (hemp) groups (1 and 2).
6. Psychoactive plants, hybrid cultivars between two narcotic groups (3 and 4).

Hillig and Mahlberg (2004) analysed the content of cannabinoids in various cannabis plants and based on geographical origins, morphological features and

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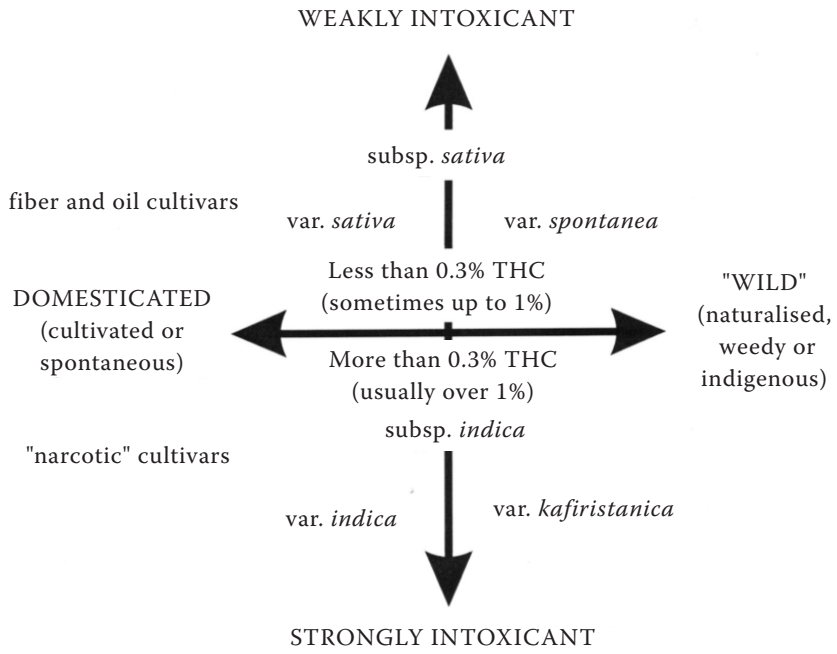

Figure 2. Cannabis chemotypes (Small and Cronquist 1976). THC – Δ^9-tetrahydrocannabinol

the supposed purpose of cultivation assigned them to the intraspecific taxa (biotypes):

Cannabis sativa "hemp biotype" – 62 plants were analysed, ranges of the dry-weight percentages of THC were measured 0.1–11.5% and CBD were measured 0.0–13.6%.

Cannabis indica "hemp biotype" – 45 plants were analysed, ranges of the dry-weight percentages of THC were measured 0.1–9.3% and CBD were measured 0.0–8.5%.

Cannabis indica "narrow-leaflet drug (NLD) biotype" – 68 plants were analysed, ranges of the dry-weight percentages of THC were measured 1.4–12.4% and CBD were measured 0.0–0.1%.

Cannabis indica "wide-leaflet drug (WLD) biotype" – 40 plants were analysed, ranges of the dry-weight percentages of THC were measured 0.1–14.7% and CBD were measured 0.0–11.0%.

All cannabis species successfully cross and produce fertile hybrids (Beutler and Marderosian 1978). *Indica* and *sativa* plants have also been found to differ in terpene and cannabinoid profiles. Thus, these chemotaxonomic markers are a promising tool for screening hybrids (Hillig 2004, Hillig and Mahlberg 2004, Fischedick et al. 2010, Elzinga et al. 2015). Zhang et al. (2018) are recommending that Cannabis should be recognised as a monotypic species typified by *Cannabis sativa* L., containing three subspecies: subsp. *sativa*, subsp. *indica*, and subsp. *ruderalis*. This proposal is based on their study focused on DNA sequence variations of cannabis plants. Also, McPartland (2018) in his work mentions that DNA barcode analysis supports the separation cannabis at a subspecies level and recognising the nomenclature of *C. sativa* subsp. *sativa* and *C. sativa* subsp. *indica*.

BIOSYNTHESIS OF CANNABINOIDS

History

Actual cannabinoid research is based on a number of major discoveries made by Professor Raphael Mechoulam and Professor Yechiel Gaoni. In the 1960's they identified the psychoactive component in *Cannabis sativa*, Δ^9-tetrahydrocannabinol, determined and described its chemical structure (Gaoni and Mechoulam 1964, Mechoulam and Gaoni 1967) and synthesised it (Mechoulam et al. 1967). Endogenous cannabinoid receptor ligands, called endocannabinoids, were identified in mammalian tissues in the 1990s. The best-known examples are anandamide (Devane et al. 1992) and 2-arachidonoylglycerol (Mechoulam et al. 1995). Endocannabinoids are derived from arachidonic acid, and membrane lipids serve as a potential source of this fatty acid (Giuffrida et al. 2001). For this reason, cannabinoids from cannabis are often referred to as phytocannabinoids to differentiate them from endocannabinoids.

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Biosynthesis of phytocannabinoids

Phytocannabinoids can be divided into two groups, neutral cannabinoids and cannabinoid acids. Diversification is based on how many carboxyl groups the molecule has, but non-enzymatic decarboxylation can occur during storage and especially at elevated temperatures when cannabis is smoked (Kimura and Okamoto 1970, Shoyama et al. 1970). Phytocannabinoids, prenylated polyketides of mixed biosynthetic origin, are synthesised from fatty acid precursors and isoprenoids. All phytocannabinoid structures contain a monoterpenic unit attached to the phenolic ring having the C3 alkylated carbon (Dewick 2002). The alkyl side chain can vary in length from one to five carbons (Figure 3) and n-pentyl is the most abundant (Elsohly and Slade 2005). Phytocannabinoids containing an n-propyl side chain are referred to as cannabivarins. Tetrahydrocannabivarin (THCV), the THC analogue with an n-propyl side chain, often occurs in *C. indica* (Hillig and Mahlberg 2004).

The starting materials for aromatic ring synthesis, including the alkyl on the third carbon (Hanuš et al. 2016), are three molecules of malonyl-CoA and one molecule of hexanoyl-CoA derived from hexanoic (caproic) acid (Dewick 2002). The hexanoyl-CoA acts as a primer for the type III polyketide synthase enzyme, also known as tetraketide synthase (TKS), which also requires the olivetolic acid cyclase enzyme (OAC) catalysing a C2–C7 intramolecular aldol condensation with carboxyl group retention to produce olivetolic acid (Taura et al. 2009, Gagne et al. 2012). These transformations can give rise to by-products such as 4-hydroxy-6-pentylpyran-2-one (PDAL), 4-hydroxy-6-(2-oxoheptyl)pyran-2-one (HTAL) and olivetol. Cannabigerolic acid (CBGA) is further derived from olivetolic acid after alkylation with a monoterpene unit, geranylpyrophosphate, with the participation of geranylpyrophosphate:olivetolate geranyltransferase (GOT) (Figure 4) (Fellermeier and Zenk 1998). Also, the (Z)-isomer of cannabigerolic acid, cannabinerolic acid (CBNRA), is synthesised to a small extent when neryl pyrophosphate is used by the GOT enzyme instead of geranyl pyrophosphate (Taura et al. 1995a). There are three acids that can be formed from CBGA and CBNRA.

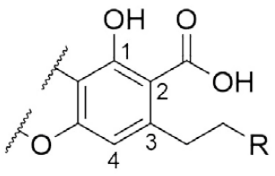

Figure 3. Structure of cannabinoids

Tetrahydrocannabinolic acid (THCA) is produced during the formation of the heterocyclic ring by the THCA synthase enzyme, which can convert CBGA or CBNRA to THCA (Figure 5) (Taura et al. 1995b). However, the low THCA synthase specificity for CBNRA compared to CBGA suggested that THCA was predominantly synthesised from CBGA. The course of this reaction is similar to that of other reactions catalysed by monoterpenic cyclases. Most of the cyclases require divalent ions such as Mg^{2+} or Mn^{2+} for their activity, but this is not the case with THCA synthase (Taura 2009). The presence of a carboxyl group in the substrate molecule is essential for the reaction because THCA synthase does not recognize neutral phytocannabinoids such as cannabigerol (CBG) as substrates (Taura et al. 2007a).

The structure of cannabidiolic acid (CBDA) is the result of a pericyclic reaction involving loss of a proton (Figure 6) (Dewick 2002). The modification is catalysed by the intramolecular oxidoreductase, CBDA synthase, which selectively favours the formation of CBDA from CBGA over its (Z)-isomer, CBNRA (Taura et al. 1996). The effects of various metal ions (Mg^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+} and Cu^{2+}) on its activity were investigated, but they did not alter the rate of catalysis. In contrast, the Hg^{2+} ion completely inhibited enzyme activity at a concentration of 2 mmol, and the chelating agent, ethylenediaminetetraacetic acid (EDTA), at concentrations up to 5 mmol showed a low positive effect on enzyme activity. Thus, CBDA synthase does not appear to require metal ions for CBGA oxidocyclization (Taura et al. 1996). CBDA synthase and THCA synthase catalyse the formation of single optical isomers at a purity of greater than 95% (Taura et al. 2007b).

Cannabichromenic acid (CBCA) is derived from CBGA by oxidation and cyclisation by cannabichromenic acid synthase (CBCA synthase) (Figure 7). CBCA is synthesised as a 5:1 enantiomeric mixture, probably because of the partial release of intermediates from the CBCA synthase active site prior to completion of the reaction (Morimoto et al. 1997). Tests of the metal ions, Mg^{2+}, Zn^{2+}, Ca^{2+} and Cu^{2+}, showed that none of them stimulated enzyme activity. Hg^{2+}, however, completely inhibited the reaction at a concentration of 1 mmol. EDTA slightly increased enzyme activity suggesting that the CBCA synthase reaction does not require metal ions (Morimoto et al. 1998).

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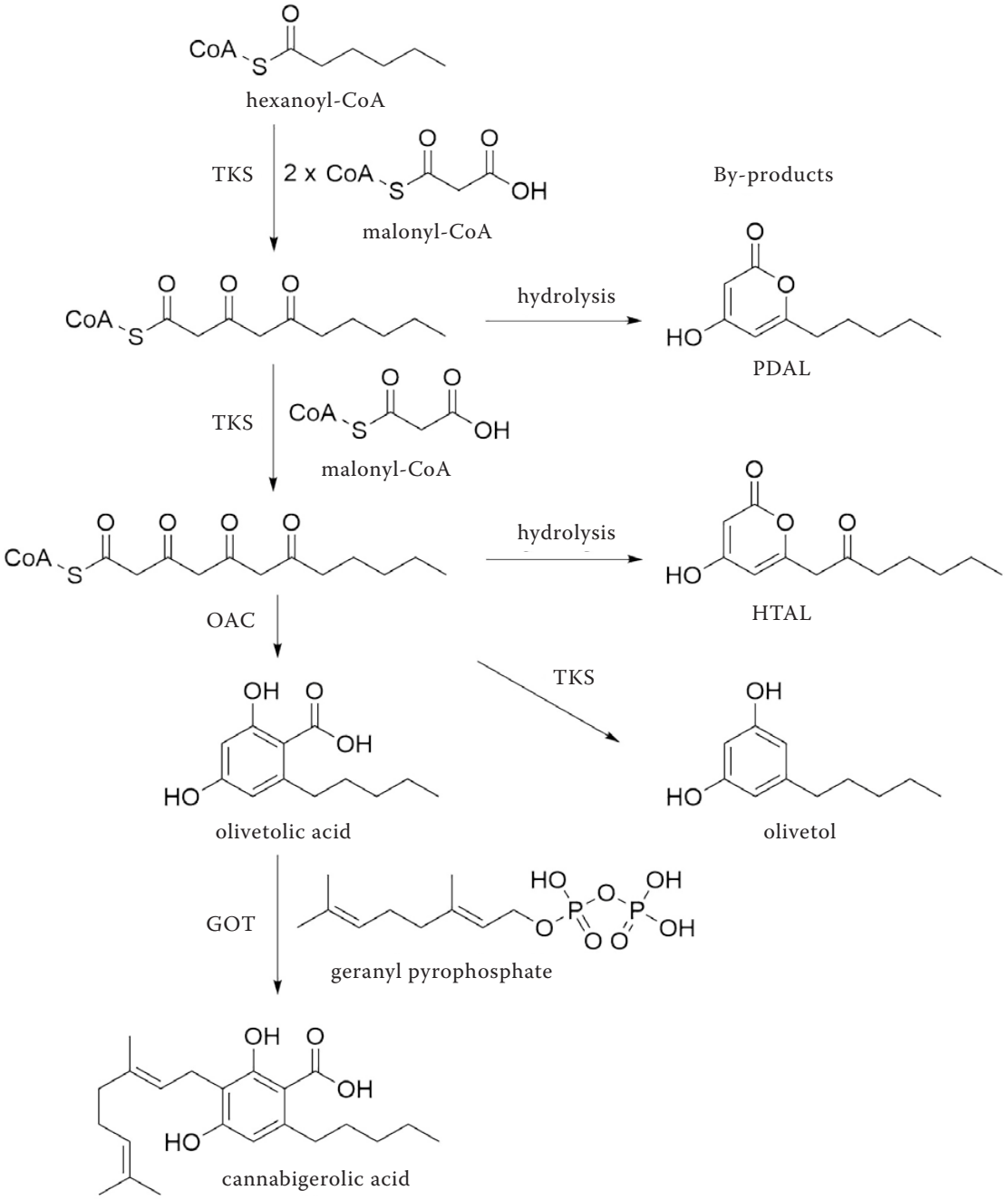

Figure 4. Biosynthesis of phytocannabinoids 1/2. TKS – tetraketide synthase; PDAL – 4-hydroxy-6-pentylpyran-2-one; OAC – olivetolic acid cyclase enzyme; HTAL – 4-hydroxy-6-(2-oxoheptyl)pyran-2-one; GOT – geranylpyrophosphate: olivetolate geranyltransferase

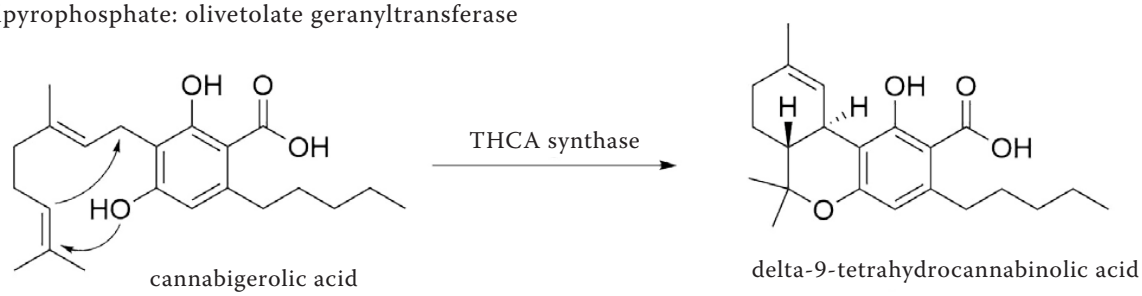

Figure 5. Δ^9-THCA (tetrahydrocannabinolic acid) synthesis

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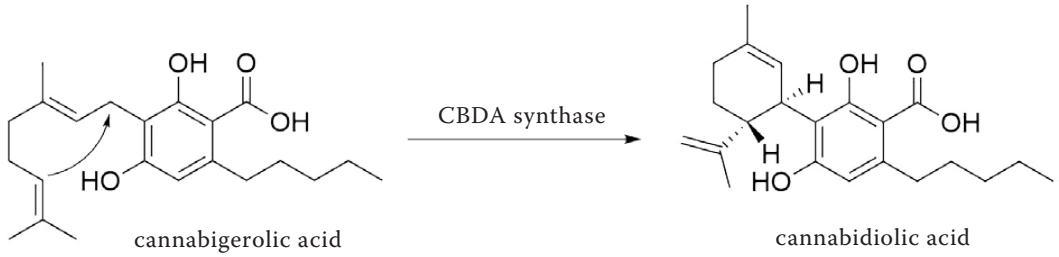

Figure 6. Cannabidiolic acid (CBDA) synthesis

Croteau (1987) discovered that all terpene cyclases require bivalent cations for their function because these metal ions are able to neutralise the negative charge on the diphosphate groups on the terpene molecules and ionise the allyl diphosphate substrate. Since CBGA does not contain a diphosphate group it is to be expected that CBCA synthase, CBDA synthase and THCA synthase have no requirement for bivalent cations. The most of the cannabinoids present in *C. sativa* can be categorised as Δ^9-tetrahydrocannabinol (Δ^9-THC), CBD, CBC, CBG, cannabinol (CBN), cannabicyclol (CBL), cannabielsoin (CBE) and cannabitriol (CBT) (Turner et al. 1980, Razdan 1986, Ross and Elsohly 1995). Δ^9-THCA, CBDA and CBCA are also sometimes called primary phytocannabinoids because other phytocannabinoids are generated from these three precursors predominantly by nonenzymatic degradative pathways.

Primary phytocannabinoids can either be decarboxylated to their neutral form (Figure 8) or converted to CBE, CBN, CBT, Δ^8-tetrahydrocannabinol (Δ^8-THC) or CBL *via* exposure to light, heat and oxygen (Figure 9). CBD can undergo photooxidation or pyrolysis to form CBE. Δ^9-THC is converted to the thermodynamically more stable Δ^8-THC when exposed to heat, or it may be degraded to CBT or CBN in the presence of oxygen (Elsohly and Slade 2005). The presence of CBT and CBN together with high levels of decarboxylated phytocannabinoids, are the chemical indicators of lengthy storage under poor conditions (Shoyama et al. 1970). The degradation rate of primary phytocannabinoids to these secondary phytocannabinoids increases with higher temperature, higher initial concentrations of primary phytocannabinoids, and with an increase in the inflorescence surface, and thus greater surface exposure to air (Milay et al. 2020). CBC in the presence of light converts to CBL-type phytocannabinoids (Elsohly and Slade 2005). Cannabivarins are generated by the same biosynthetic pathways from cannabigerovarinic acid (CBGVA), a homologous CBGA precursor (Shoyama et al. 1984). The cannabinoid profile in Cannabis undergoes rapid changes in the early stages of growth (Potter 2014). CBDA and THCA synthases have very similar catalytic rates (k_{cat} = 0.19/s and 0.20/s) and affinity (K_M = 134 µmol and 137 µmol) for cannabigerolic acid (Taura et al. 1995b, 1996). The CBCA synthase, however, shows a lower Michaelis constant (K_M = 23 µmol) as well as a higher catalytic rate (k_{cat} = 0.04/s). In the early stages of cultivation, where CBGA is still present at low concentrations, CBCA synthesis predominates (Morimoto et al. 1998). However, as the CBGA concentration increases over time, the efficacy of THCA and CBDA biosynthesis increases, and these molecules soon outweigh the CBCA concentration. At later stages of growth, CBGA synthesis slows and its relative proportion in the phytocannabinoid profile is gradually reduced (Potter 2014).

CULTIVATION

In European countries, most cannabis is grown for industrial purposes (Zuk-Golaszewska and Golaszewski

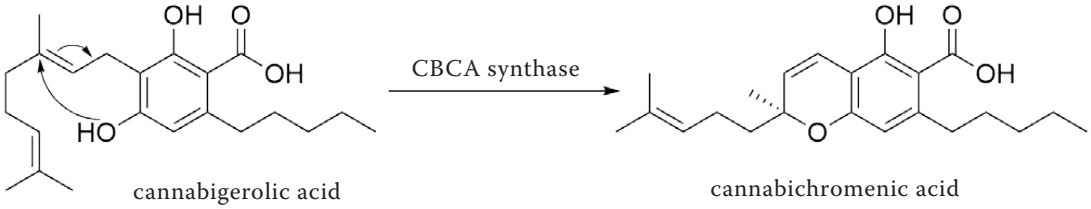

Figure 7. Cannabichromenic acid (CBCA) synthesis

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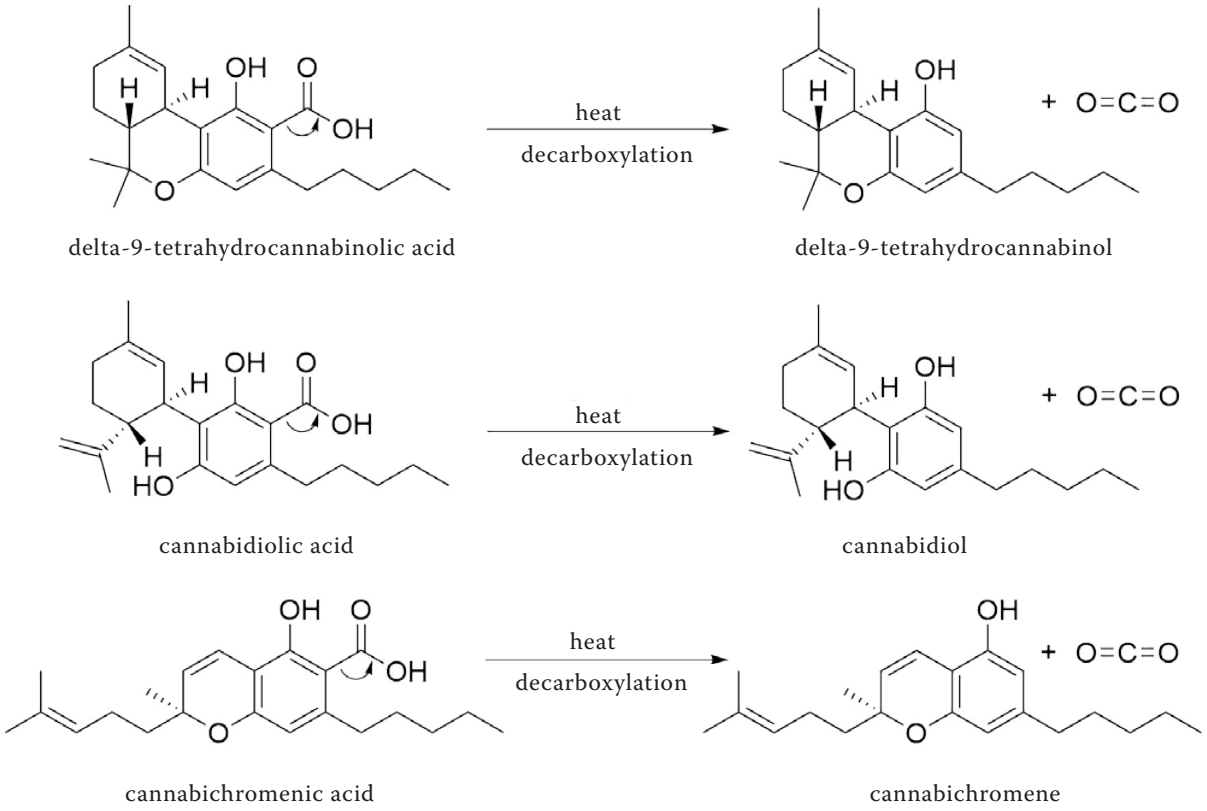

Figure 8. Decarboxylation of primary phytocannabinoids

2018) such as hemp fibers (Pickering et al. 2007), seeds as a source of oil (Mölleken and Theimer 1997, Kriese et al. 2004) and protein (Patel et al. 1994). For this reason, the procedures for hemp cultivation are well known, while the growth factors affecting cannabis cultivation for medical purposes are poorly understood (Zuk-Golaszewska and Golaszewski 2018).

Indoor or outdoor medical cannabis cultivation?

The conditions under which cannabis plants are grown for drug production is subject to more stringent protocols relating to the content and type of the active phytocannabinoids. Among the factors influencing the composition and yield of phytocannabinoids are the genotype of the plant, the growing conditions, maturity at harvest time, storage and handling (Potter 2014).

It is much more efficient to grow medical cannabis plants in a greenhouse where light, temperature and humidity can be controlled. Until recently, this method of cultivation was used mainly by illegal cannabis growers (Drugs 2009). Outdoor cultivation is less expensive, but the variability of the environment makes it almost impossible to obtain a high-potency, homogeneous product. Cannabis that is grown outdoors is also at greater risk from pests and plant diseases (Potter 2014). Cannabis entrepreneurs now use sophisticated indoor cultivation methods with automated control of lighting and photoperiod, temperature, ventilation and irrigation, and complex systems for providing nutrients. However, much of the information on indoor cannabis production is still obtained from anecdotal sources (Vanhove et al. 2011). Current data on the influence of photoperiod and even light spectrum allow indoor growers to regulate such aspects as leaf and shoot growth and time of vegetation cycle and thus achieve several growth cycles per year (Farag and Kayser 2015). Three to six harvests per year (six harvests per year is the maximum, and in this case, you have to skip the vegetative phase) can be attained by applying modern controlled growing practices (Leggett 2006).

Hydroponics *versus* soil

Indoor cannabis cultivation can be accomplished in several ways, but primarily either in soil or in soilless culture using hydroponic media. Hydroponic

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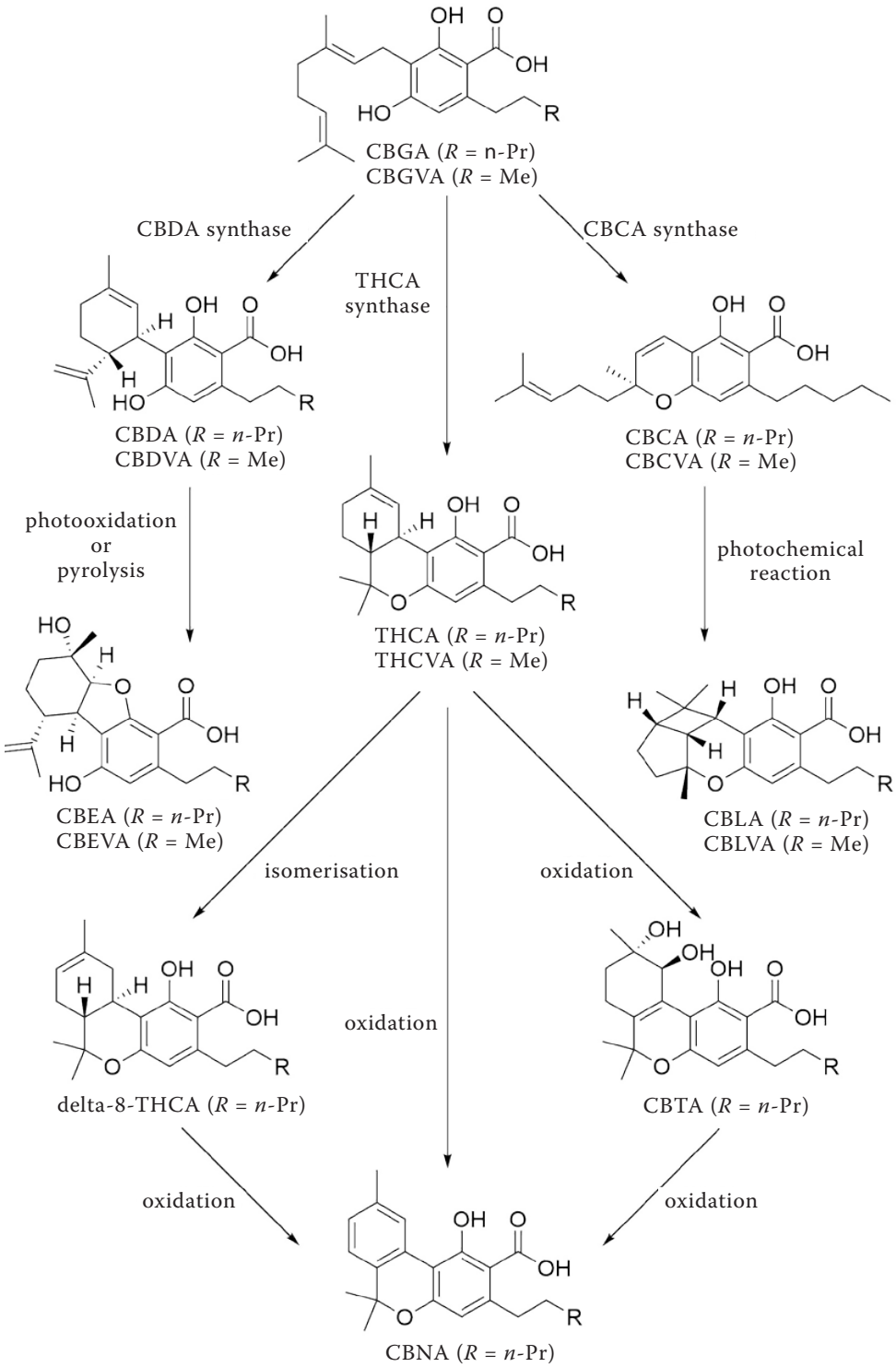

Figure 9. Biosynthesis of phytocannabinoids 2/2. CBGA – cannabigerolic acid; CBGVA – cannabigerovarinic acid; CBDA – cannabidiolic acid; CBDVA – cannabidivarinic acid; CBCA – cannabichromenic acid; CBCVA – cannabichromevarinic acid; THCA – tetrahydrocannabinolic acid; THCVA – tetrahydrocannabivarinic acid; CBEA – cannabielsoin acid; CBEVA – cannabielsovarinic acid acid; CBLA – cannabicyclolic acid; CBLVA – cannabicyclolvarinic acid; CBTA – cannabitriolic acid; CBNA – cannabinolic acid

cultivation has become increasingly popular among growers. A soilless media such as mineral wool, coconut fibers, perlite or expanded clay are used while nutrients provided by solutions are applied directly

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to the roots (Vanhove et al. 2011). The conventional type of cultivation is in soil with fertilisers applied through irrigation or by mixing with the soil substrates. Potter (2014) found that there was no increase in phytocannabinoid potency or in biomass under hydroponic conditions compared to standard soil cultivation, and the hydroponic system was more complicated and difficult to operate and maintain.

Vegetation cycle of cannabis

Cannabis is a short-day plant, naturally blooming in autumn, and the induction of flowering is regulated by specialised photoreceptor proteins called phytochromes. Therefore, the effect of photoperiod must be taken into an account in indoor cultivation (Halliday and Fankhauser 2003). The vegetative phase lasts from 2–4 weeks after rooting the clones or germinating the seeds (De Backer et al. 2012). The relative humidity in this phase should be from 70% to 80% with a temperature from 21 °C to 28 °C (Chandra et al. 2008). The generative phase is induced by shortening the photoperiod to 12 h light and 12 h dark. The first flowers should appear about one week after the reduction of the light period. The development of stems and leaves gradually slows down and stops after three weeks of this photoperiod, while the flowers continue to develop over the next 8 weeks (De Backer et al. 2012). The monitoring of 200 high THC cannabis varieties showed that the average flowering time with 12 h light period was 57 days, and 88% of the plants flowered between 7 and 9 weeks (Carpentier et al. 2012). The recommended temperatures are similar to the previous phase from 21 °C to 28 °C. However, humidity should be lowered to 40% over the generative phase to reduce the risk of fungal diseases (Vanhove et al. 2011, 2012).

Effect of CO_2 concentration

In order to prevent mold, a dry environment and constant air circulation should be ensured in indoor cannabis growing rooms, either from outdoor ventilation with filters or by indoor fans. It is also recommended to increase the concentration of CO_2 during the light phase of the day (cycle) to improve photosynthesis, plant growth and thus increase biomass yield (Kimball 1983, Wheeler et al. 1996, Chandra et al. 2008, 2011). Elevated CO_2 concentration can improve the assimilation of carbon, thereby accelerate plant growth and potentially improve productivity (Kimball 1983). There is a close correlation between plant yield and photosynthesis rate because more than 90% of plant dry matter is derived from photosynthetic CO_2 assimilation (Zelitch 1975). However, the improved level of plant photosynthesis and growth appear to be species- and variety-specific (Minorsky 2002).

Wang et al. (2008) investigated the effects of standard (370 ppm) and high (700 ppm) CO_2 concentrations on photosynthesis tolerance to acute heat stress (daily growth temperature was increased by 15 °C every day for 4 h) in cool-season and warm-season of C3 plants. High CO_2 concentration increased the cool-season and warm-season C3 plants tolerance of photosynthesis to acute heat stress. Hamilton et al. (2008) further elaborated the previous idea and concluded that the effects of growth temperature on photosynthetic thermotolerance between C3 and C4 plants are different and affected by the state of acclimatisation of the plants. A high concentration of CO_2 (700 ppm) increases the thermotolerance of C3 plants photosynthesis, except for C3 plants grown at the supra-optimal (5 °C above optimal) growth temperature, then increased CO_2 may provide no advantage or even reduce photosynthesis. On the other hand, increased CO_2 often reduces the photosynthetic thermotolerance of C4 plants at both optimal and supra-optimal growth temperatures.

Chandra et al. (2011) performed experiments directly on cannabis and showed that increasing CO_2 concentration from 390 ppm to 700 ppm increased the rate of photosynthesis in different varieties of *Cannabis sativa* by 38–48% and improved efficiency of water uptake.

Artificial light

To achieve optimal biomass and phytocannabinoid production, artificial lighting must meet certain parameters. These include light intensity in lumens per m^2 (lux units) and radiation intensity in watts per m^2 and the wavelength. Wavelength is particularly important because plants require different wavelengths of light during the growth. In the vegetative (roots and shoots) phase, the light should be 420–460 nm which corresponds to blue light, which promotes phototropism and growth hormone production in the plants. In the flowering phase, a red spectrum (600–680 nm) that is well absorbed by chlorophyll is best (Mahlberg and Hemphill 1983). For indoor cannabis cultivation, fluorescent T-5 lighting, metal-halide lamps (MH), high-pressure sodium lamps (HPS) for the growth and light-emitting diodes (LED),

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high-pressure sodium lamps (HPS) for the generative phase are most commonly used (Sweet 2016). These lamps differ in the composition of the inside gases, and they produce the light of different wavelengths.

The optimal intensity of illumination. The experiments of Potter and Duncombe (2012) showed positive relationship between the intensity of illumination and amount of biomass harvested. They determined three zones with elevated illumination energy 270, 400 and 600 W/m^2. Five plants of each variety were placed in each of the three zones at a density of 10 plants/m^2. In the growth rooms, daily average temperatures were maintained at 25 ± 2 °C. A constant supply of fresh air kept CO_2 concentration in the environment between 350 ppm and 390 ppm. Irradiance levels at the surface of the plant canopy were measured using a hand-held light meter determined the photosynthetically active radiation 80, 120 and 180 W/m^2 according to variants. Within plants growth, the lamps were kept at a constant distance from the cannabis canopy. The greatest harvest was achieved at 600 W/m^2 of the illumination intensity. Furthermore, the THC contents in the leaves and inflorescences of the mentioned variants were measured, but no significant increase in the concentration of THC was recorded with an increase of light intensity. Toonen et al. (2006) also reported that plants grown under 600 W lamps achieved higher yields than plants grown under 400 W lamps.

Decreasing tendency of plants to convert light energy into biomass with increasing levels of radiation is probably due to the fact, that plants have a limited ability to use light for photosynthesis. Under low light conditions, plants normally show an initial linear increase in the rate of photosynthesis and thus a tendency to convert light energy into biomass in response to increasing irradiation. However, under brighter conditions, the growth rate slows as chloroplasts become more and more saturated with light (Evans et al. 1993, Ögren and Evans 1993). This has also been proven on cannabis. The rate of increase in photosynthetic activity went down rapidly when irradiation levels rose above 100 W/m^2 of photosynthetically active radiation. Since 300 W/m^2 of photosynthetically active radiation, almost no increase in photosynthetic activity has been observed (Lydon et al. 1987).

HPS lamps *versus* LED. Magagnini et al. (2018) concluded that HPS-lit plants were higher and had a larger amount of dry matter than LED-lit plants. Conversely, plants under LED fixtures contained higher levels of CBD and THC than under the HPS. Namdar et al. (2019) also found out significant increase in concentration of CBGA in the inflorescences that flowered under LED illumination, with CBGA:THCA ratio of 1:2 as opposed to 1:16 when grown under HPS. Because of the high level of illumination, it was necessary to install a ventilation fan for cooling to the optimum temperature for photosynthesis of 25 °C to 30 °C (Bazzaz et al. 1975). A more efficient alternative is to use banks of LEDs that produce relatively little heat (Bessho and Shimizu 2012). LEDs do not consume much energy, do not require ballasts, and produce only a small amount of heat compared to high intensity discharge lamps. LEDs are compact, have long lives, very good wavelength specificity, relatively cool radiating surfaces, and linear photon output with electrical input current (Massa et al. 2008).

NUTRITION

In the area of plant nutrition for medical cannabis production, there is currently a lack of experimental data in the literature (Caplan et al. 2017a). It is known that the content of cannabinoids in leaves gradually decreases from top to bottom of the hemp plant (Hemphill et al. 1980) and from the literature about hemp cultivation can be deduced that nutrient application can affect the final cannabinoid content of the plants as well as their total yield. This suggests that nutrition could play a similar role for medical cannabis grown under controlled conditions. However, cannabis for hemp production has been selectively bred to produce fiber and is therefore likely to have slightly different nutrient needs than cannabis grown for medicinal purposes. The hemp crop is also grown in the field and not indoors (Hillig and Mahlberg 2004, Van Bakel et al. 2011, Amaducci et al. 2015).

Acceptable forms of individual essential nutrients are divided by Barker and Pilbeam (2015) into two groups according to plant needs, namely macronutrients: nitrogen (NO_3^-, NH_4^+), phosphorus ($H_2PO_4^-$, HPO_4^{2-}), potassium (K^+), calcium (Ca^{2+}), sulfur (SO_4^{2-}), magnesium (Mg^{2+}), and micronutrients: iron (Fe^{2+}, Fe^{3+}), chlorine (Cl^-), manganese (Mn^{2+}), zinc (Zn^{2+}), copper (Cu^+, Cu^{2+}), boron (H_3BO_3, $H_2BO_3^-$), molybdenum (MoO_4^{2-}) and nickel (Ni^{2+}).

Macronutrients

Nitrogen, phosphorus and potassium (NPK). It is assumed that the nitrogen content in the vegetative parts of

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the hemp plant positively correlates with the THC content (Haney and Kutscheid 1973). Thus, older leaves contain less THC than younger leaves because they contain less nitrogen. In contrast, high nitrogen levels in applied nitrogen fertilisers reduce the THC content of the hemp leaves (Bócsa et al. 1997). For example, good hemp production requires optimum soil nitrogen levels in the range of 50–200 kg/ha (Vera et al. 2004, Aubin et al. 2015), but these recommendations are not applicable for hydroponic or soil cultivation where studies on indoor cannabis cultivation indicated nitrogen fertilisation should be provided in the range of 190–400 mg N/L. This value has also been reported for nitrogen supplementation of organic, greenhouse-grown tomatoes (Zhai et al. 2009, Surrage et al. 2010).

Hemp growth and an increase in THC content were positively correlated with soil P content (Coffman and Gentner 1977). A negative relationship has been reported for CBD content in leaf tissue relative to available P. Hemp grown on soils depleted of P showed an increased CBD content (Coffman and Gentner 1975). Conversely, phosphorus enhancement did not show any positive effect on THC, CBD, CBN or CBG concentrations in buds from the top of the medical cannabis plants (Bernstein et al. 2019b).

Saloner et al. (2019) investigated response of medical cannabis to different potassium supply in vegetative growing phase. The results show that the response to nutrition is highly dependent on the genotype. Plants in this study were exposed to five different levels of K supply (15–240 ppm). Generally, both cultivars showed increased K concentration in all plant parts with increased K supply. Insufficient K dose for optimal growth and function was the lowest tested supply 15 ppm of K. Also, the highest dose proved excessive and damaging effect to development for one of the two tested genotypes. Similarities proven at both genotypes were in trends of accumulation and uptake. Results demonstrated competition between K and Ca with Mg uptake and no effect on P and N uptake except in the K deficiency range. Potassium supply showed only little effect on micronutrient accumulation in the plant shoot which was similar for both cultivars.

In contrast, no significant effect on hemp biomass and THC was observed in relation to different doses of N and K (Coffman and Gentner 1977). According to Hanuš and Dostálová (1994), various combinations of selected macroelements (N, P, K) in hemp culture can significantly affect the type of phytocannabinoids present and their individual contents. One of a few available sources of scientific literature dealing directly with this issue is the article by Caplan et al. (2017a, b), who reported a concentration of 389 mg N/L as optimal during the growth phase for maximum yield. The ratio of the basic macroelements (N, P, and K) in the vegetative period was 4:1.3:1.7. After making the calculations for P and K, we obtained values of 126 mg P/L and 165 mg K/L. In the generative phase, 212–261 mg N/L was the optimal amount. A nitrogen concentration of 283 mg N/L gave the maximum yield of inflorescence and biomass, but the concentrations of phytocannabinoids in the dried product was lower. The ratio of N, P, and K in the generative period was set at 2:0.87:3.32. Therefore, an initial concentration of 283 mg N/L, would require 123 mg P/L and 470 mg K/L. The plants tested were propagated from 17 day-old cuttings, which were fertilised with a solution of the indicated concentration for the following 21 days of vegetative growth. Another study has proved sensitivity of phytocannabinoids metabolism to mineral nutrition. The results presented by Bernstein et al. (2019b) show that increased treatment of inorganic NPK increased levels of CBG in flowers by 71% and decreased levels of CBN in flowers by 38% compared to a control treatment. Plants in the control variant were cultivated in potting mixture with fertigation. Concentration of dissolved nutrients in the control variant was as follows: 65 ppm N (1:2 ratio of NH_4^+/NO_3^-), 17 ppm P (40 ppm P_2O_5), 90 ppm K (108 ppm K_2O). Micronutrients were supplied chelated with EDTA at concentration of 0.4 ppm Fe, 0.2 ppm Mn, and 0.06 ppm Zn.

The rest of macronutrients. The magnesium cation content in soils is relatively mobile and its concentration in plants, especially in leaves, is high because it is a component of chlorophyll. The negative correlation between this metal and copper results from the fact that the radii of their ions are similar and both ions can compete for the same binding sites. The content of Δ^9-THC and CBD in hemp leaves decreases with increasing Mg concentration in the soil. The Δ^9-THC content in leaves is positively correlated with the ratio of accessible Ca/Mg in soil. CBD is negatively correlated with available Ca/Zn and Mg/Cu ratios. Positive correlations of magnesium with Δ^8-THC have been reported with the hypothesis that this nutrient may be cofactor in the enzyme responsible for its production (Coffman and Gentner 1975, Pate 1994, Radosavljevic-Stevanovic et al. 2014).

Micronutrients

Similar results have been seen for micronutrient requirements. Positive correlations of iron with

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Δ^8-THC have been reported with the hypothesis that this nutrient may be cofactor in the enzyme responsible for its production (Pate 1994, Radosavljevic-Stevanovic et al. 2014). CBD content in hemp plants is decreasing with increasing iron concentration (Radosavljevic-Stevanovic et al. 2014). The negative correlation of iron (Fe) and chromium (Cr) with CBD can be explained because the catalase responsible for the decomposition of hydrogen peroxide from the CBDA synthase reaction is a member of the class that contains four heme iron groups. Hydrogen peroxide is strongly sterically hindered upon entry into the heme cavity where the first step of catalysis takes place. Transferring a proton from an oxygen atom to a hydrogen peroxide molecule, and then to a second oxygen atom extends and polarises the O-O bond, which eventually decays heterolytically. The first oxygen atom of the hydrogen peroxide molecule is coordinated with a heme center, which releases water and creates an $O=Fe^{(IV)}$-$enzym^{(+)}$ heme radical. The radical then quickly breaks down by electron transfer, removing the radical electron from the porphyrin ring, which remains unchanged. During the second step, in a similar two-electron transmission reaction, the $O=Fe^{(IV)}$-$enzym^{(+)}$ reacts with a second molecule of hydrogen peroxide to form the parent molecule $Fe^{(III)}$ – enzym, water, and molecular oxygen (Boon et al. 2007, Vlasits et al. 2010).

Proposed reaction mechanism:

$$H_2O_2 + Fe^{(III)}\text{-enzyme} \leftrightarrow H_2O + O=Fe^{(IV)}\text{-enzyme}^{(+)}$$

$$H_2O_2 + O=Fe^{(IV)}\text{-enzyme}^{(+)} \leftrightarrow H_2O + Fe^{(III)}\text{-enzyme} + O_2$$

Fe-enzyme represents the center of heme iron attached to the rest of the enzyme.

The transition state, $O=Fe^{(IV)}$-$enzyme^{(+)}$ is energetically unstable, so these reactions are disadvantageous (Boon et al. 2007, Vlasits et al. 2010). Although chromium is not important for plant growth, its negative correlation with CBD is explained by the fact that Fe and Cr occur together in nature as a complex oxide (Radosavljevic-Stevanovic et al. 2014).

The concentration of CBN and Δ^9-THC in hemp plants can be influenced by the amount of manganese (Radosavljevic-Stevanovic et al. 2014). A positive correlation of manganese with CBN has been reported (Pate 1994, Radosavljevic-Stevanovic et al. 2014). THCA synthase, which catalyses the oxidative cyclisation of CBGA to THCA, contains a flavin adenine dinucleotide (FAD) prosthetic group that is reduced to $FADH_2$. Molecular oxygen is required to re-oxidise the $FADH_2$ to FAD, with the formation of hydrogen peroxide in a 1:1 molar ratio to the resulting THCA as a by-product of the reaction (Flores-Sanchez and Verpoorte 2008, Shoyama et al. 2012). CBDA synthase also contains FAD that is reduced to $FADH_2$ with release of H_2O_2, but the reaction differs from THCA synthase in the proton transfer step (Figure 10) (Taura et al. 2007a). It is estimated that about 1% of oxygen in plants is used to form reactive oxygen species in different subcellular locations with hydrogen peroxide being the most abundant. Hydrogen peroxide causes oxidative damage to cells that can lead to apoptosis (Quan et al. 2008), and plants have evolved efficient ways of eliminating toxic levels of H_2O_2. Catalase is a peroxidase enzyme found in all oxygen-using organisms that rapidly converts H_2O_2 to water and oxygen. There are three types of catalase and the non-heme form utilises manganese (Mn^{3+}) in its catalytic center that is reduced to Mn^{2+} during the decomposition of H_2O_2 to water and oxygen. Mn^{2+} can then react with more peroxide and be converted back to Mn^{3+} according to the following equations:

$$2Mn^{3+} + H_2O_2 \leftrightarrow 2Mn^{2+} + O_2 + 2H^+$$

$$2Mn^{2+} + H_2O_2 + 2H^+ \leftrightarrow 2H_2O + 2Mn^{3+}.$$

Both reactions are energetically advantageous ($\Delta G < 0$). The correlation between manganese and CBN is also positive since CBN is the primary THC degradation product (Wu et al. 2004).

Bernstein et al. (2019a) describes translocation of individual macro and microelements in relation to individual plant parts' age. The work also describes, inter alia, the distribution of cannabinoids in the plant. The research shows that the concentration of cannabinoids increases with the height of the plant and the highest concentration can be found in flowers and inflorescence leaves. The concentration found in fan leaves is about 1/10 the concentration found in flowers. The distribution of mineral nutrients between plant organs shows a typical uptake and translocation in the plant. Lower concentrations of N, P, K, and higher Ca in fan leaves compared to inflorescence supports physiological findings that the fan leaves are older than the inflorescence leaves.

pH value

Suggested optimal pH range of nutrient solution is between 5.5–6.5. pH is important because it affects the availability and absorption of nutrients needed

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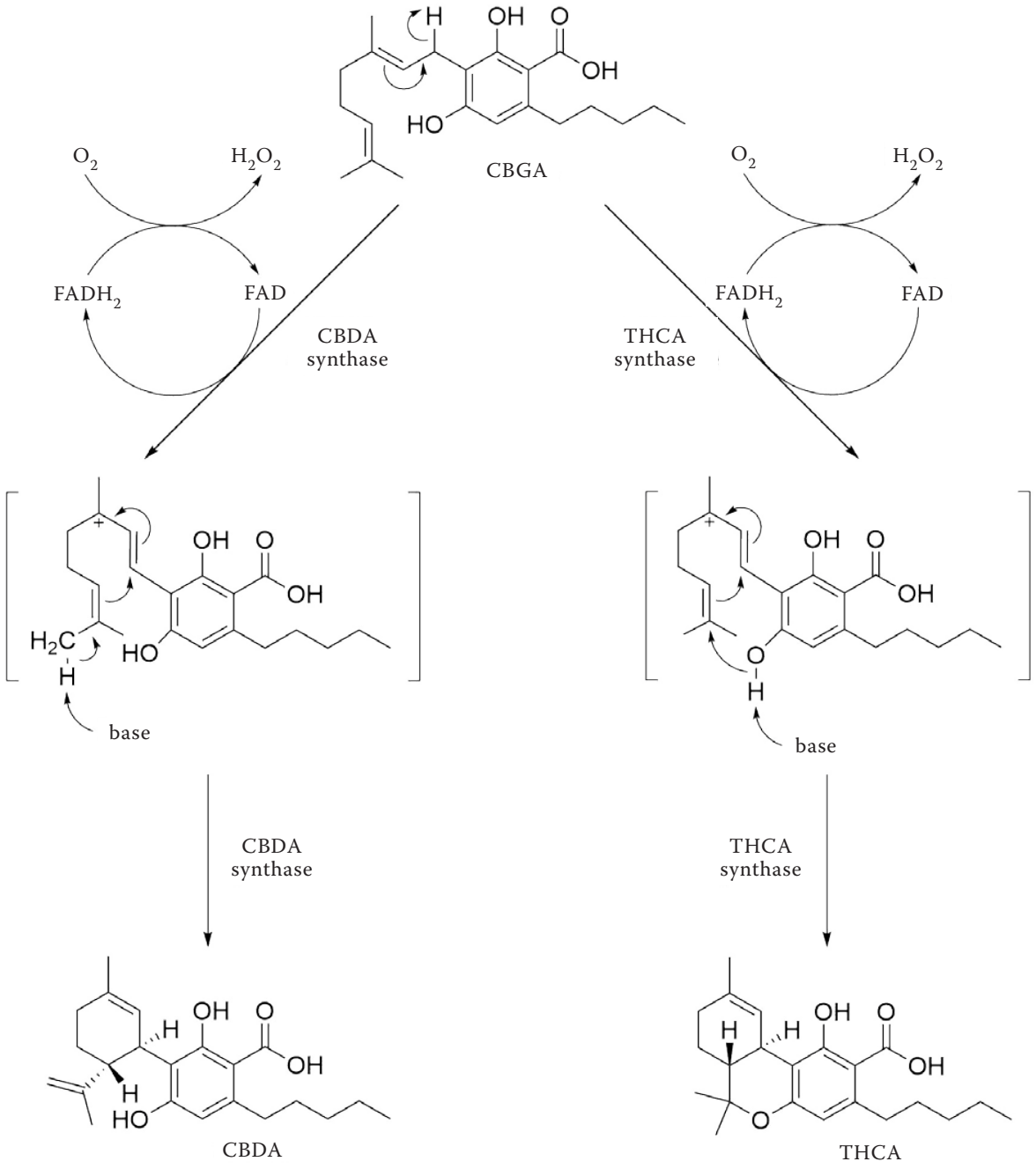

Figure 10. Reaction mechanism of tetrahydrocannabinolic acid (THCA) synthase and cannabidiolic acid (CBDA) synthase. CBGA – cannabigerolic acid; FAD – flavin adenine dinucleotide

for plant growth. In hydroponic culture, the recommended pH range is 5.5–6.0 and the maximum absorption of nutrients is usually at pH 5.8 (Velazquez et al. 2013). In growing substrate, a pH range of 5.8–7.2 is recommended and the maximum absorption of essential nutrients is typically at pH 6.5. When the pH falls below this range, many macronutrients are less available and macronutrient deficiencies can be developed. When pH values rise above this range, many micronutrients will not be available for the plant uptake causing micronutrient deficiencies (Caplan et al. 2017a). These authors also mention the need for further research to confirm the optimal range of pH for multiple cannabis varieties.

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Plant biostimulants

A plant biostimulant is any substance, micro-organism strain or mixture of both applied to plants to increase tolerance to abiotic stress, nutritional efficiency or crop quality characteristics, regardless of its nutrient content. Seven main biostimulant categories were proposed: humic and fulvic acids, protein hydrolysates, seaweed and botanical extracts, chitosan and biopolymers, beneficial bacteria, beneficial fungi and beneficial minerals (Du Jardin 2015).

Humic and fulvic acids in cannabis nutrition. Humic substances are natural components of soil organic matter. It is a mixture of heterogeneous compounds originally classified according to their molecular weights and solubility into humins, humic acids and fulvic acids (Du Jardin 2015).

Humic acid supplementation had a positive effect on cannabis in the case of the height of cannabis plants, the chlorophyll content and the efficiency of photosynthesis, especially immediately after the period of water stress (Da Cunha Leme Filho et al. 2020).

According to the current literature, the effect on phytocannabinoids is rather negative. Bernstein et al. (2019b) mentioned that nutritional supplements such as humic acids significantly reduced spatial variability of cannabinoids throughout the plant parts. This increased uniformity came at the expenses of THC and CBD content which was reduced by 37% and 39% respectively in the top parts of plants. The decrease of THC has been associated with an additional trend of CBN increasing. This was probably due to the accelerated degradation of cannabinoids in the plant parts with their high concentration.

Other biostimulants in cannabis nutrition. Conant et al. (2017) demonstrated that microbial biostimulant Mammoth P™ promoted cannabis growth during the blooming phase. Lyu et al. (2019) hypothesised that future research will show that plant growth-promoting bacteria can affect the accumulation of phytocannabinoids, increase inflorescence yields, protect against plant pathogens by producing antimicrobial compounds and reduce the impact of abiotic stresses.

CONCLUSIONS AND FUTURE PERSPECTIVE

Based on the above information, it can be stated that quality of medical cannabis biomass, spectrum and concentration of phytocannabinoids can be influenced by cultivation conditions as well as nutrition during cultivation.

For the cultivation of medical cannabis, due to safety reasons, unpredictable environmental influences and required homogenity of harvest, indoor cultivation is definitely a better option because optimal growing conditions can be set and cannabis can be harvested from three to six times per year. Of the growing conditions, artificial light, the level of CO_2 concentration and the humidity of the surrounding environment influence the harvest quantity and quality the most. It is very important to choose the right combination of all mentioned conditions because they affect each other.

There is currently only a few experimental data on the medical cannabis nutrition, so most of this information is based on the hemp cultivation, which was bred for fiber production rather than inflorescence. However, it can be concluded from the current literature the concentration and spectrum of individual macronutrients, micronutrients and plant biostimulants in plant nutrition has a fundamental impact on biomass formation, spectrum and amount of medical cannabis cannabinoids.

In the future, the effects of nutrient ratios and availability can reasonably be expected to be one of the main factors influencing the content and type of cannabinoids in medical cannabis plants, separate from genotype and microclimate. These issues should be explored through further experiments, which will certainly be beneficial because of growing interest in the phytocannabinoids development in public and commercial spheres. Future technical research in this area should focus on possible new indoor medical cannabis cultivation techniques or the automation of existing cultivation technologies to facilitate work.

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