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Review Article

BIOLOGY AND ECOLOGY OF GENUS *CANNABIS*: GENETIC ORIGIN AND BIODIVERSITY. *IN VITRO* PRODUCTION OF CANNABINOIDS

Vassilevska-Ivanova R.

Institute of Plant Physiolgy and Genetics, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria

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Abbreviations: BLD – broad leaf hemp; CBD – cannabidol; 2,4-D – 2,4-dinitrophenoxy acetic acid; IAA – indole-3-acetic acid; K – kinetin; NAA – 1-naphthalene acetic acid; NLH – narrow leaf hemp; THC – delta-9-te trahydrocannabidol.

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TAXONOMY

Cannabis sativa L. is the botanical name and Latin binomial of hemp. Marijuana (marihuana) is colloquial name for dried leaves and flowers of cannabis varieties rich in THC (1 to 20% Δ^9-THC). Hashish is an Arabic name for cannabis resin or compressed resin glands, containing 5 to 20% Δ^9-THC. Nowdays debates about cannabis are not confined to its value as a medicine or to its possible hazards as a recreational drug. Something much more fundamental has been engaging the experts for years: its taxonomy (Schulters et al., 1974;

*Corresponding author: ru_vas_bg@yahoo.com

Small and Conquist, 1976; Hilling, 2005; McPartland, 2018). Are all the plants belonging to the genus *Cannabis* mere varieties of a single species-or is it correct to recognise at least three separate species?

In his original 1753 classification, Carl Linnaeus identified just one, *Cannabis sativa*. The term *sativa* means "cultivated" and describes the common hemp plant grown widely across Europe in during its life cycle. *C. sativa* is native to Europe and western Eurasia where it has been grown for millennia as a fiber and seed crop, and was introduced to the New World during European colonization (Clarke and Merlin, 2013). The first indication of dissent came in 1785 when another eminent biologist, Jean-Baptiste Lamarck, was given some plant specimens collected in India. On the basis of several characteristics including their firm stems, thin bark, and the shape of their leaves and flowers, Lamarck felt that they should be distinguished from *C. sativa*. Accordingly he invoked a new species, *C indica*. *Cannabis indica*, meaning the *Cannabis* from India where the first samples of the plant reaching Europe originated. *C. indica* is native to eastern Eurasia and was spread by humans around the world primarily as a source of psychoactive

THC (Small, 2015). *C. indica* is used for marijuana and hashish production, but in many regions of eastern Asia it has a long history of cultivation for its strong fibers and nutritious seeds. Compared to the essential oil of *C. indica* varieties, *C. sativa* produces less quantity and variety of terpenes, which are of importance in the efficacy of *Cannabis* medicines (Small, 2007). *C. sativa* represents a very small portion of the genetic diversity seen in *Cannabis* worldwide, and it is not divided into subspecies based on differing origins and uses like *C. indica* (Gould, 2015; Clark and Merlin, 2016; McPartland, 2018)

The third and least well founded species is *C ruderalis*. This was the name that a Russian, Janischevsky, gave to the cannabis plants he found growing in the south eastern central region of his country. The differences he noted were mostly in the size, shape, and casing of the seeds. And even Janischevsky himself seems not to have been totally convinced that these justified a new species (McPartland, 2018).

Since the 1960s taxonomists have championed several different naming systems. Many preferred a three species concept by recognizing*C. ruderalis* as a wild species possibly ancestral to both *C. sativa* and *C. indica*. Others chose to reduce *C. indica* and *C. ruderalis* to subspecies or varieties of a single species *C. sativa*. In the late 1970s markedly different appearing hashish varieties were introduced to the West from Afghanistan and considered by some to be the true *C. indica* and by others as a fourth species *C. afghanica*, while all the other drug varieties were held to be members of *C. sativa* following the single species model. By the dawn of the new millennium confusion and disagreement reigned, but better science would prevail (Gould, 2015)

Hillig grouped *C. indica* varieties into four subspecies—three based on their diverse morphological and biochemical traits *(C. indica ssp. indica, C. indica ssp. afganica, C. indica ssp. chinensis),* and another characterized largely by its spontaneous growth habit *(C. indica ssp. Kafiristanica) (Hilling and Mahlberg, 2004; Hilling 2005).* Presently, almost all modern drug *Cannabis* varieties are hybrids between members of two *C. indica* subspecies: subspecies *indica*, representing the traditional and geographically widespread NLD landrace marijuana varieties, and subspecies *afghanica*, representing the geographically limited BLD hashish landraces of Afghanistan. It is through combining landraces from such geographically isolated and genetically diverse populations that the great variety of modern-day hybrid recreational and medical *Cannabis* varieties blossomed.

Cannabis is open-pollinated, with male and female flowers borne on separate plants, and therefore to produce a seed usually two plants must be involved. Random combinations of alleles and accompanying variation are to be expected. *Cannabis* landrace varieties are a work in progress (Hilling, 2005; Small, 2015). They are maintained by repeated natural and human selection *in situ*—nature selecting for survival and humans selecting for beneficial traits—and without persistent human selection and maintenance they drift back to their atavistic, naturally selected survival level.

The landraces strains of Cannabis are the native, indigenous or heirloom strains found on every continent of the planet. A landrace strain is pure, never crossed and

always grown in its natural environment: this isolation and the resulting inbreeding means these varieties are highly stable and exstremely vigorous. In most cases, the real landraces are found within poor, isolated rural communities in remote areas of third world countries (McPartland, 2018). Landraces are always dominant plant in their own environment and always overpower any "intrusion". A landrace results from the plant's natural adaptation to the environment and from the thorough selection made by the grower. Their gene pool is still large because they reproduce through pollination out in the open and many males and female take part in the process, resulting in a high genetic variability. In spite of their heterogeneity, Landraces share many morphological traits that distinguish them as a group, for growers make selections according to their objectives. They are regarded as the first step in cannabis domestication. These locale-specific varieties of cannabis "landraces" are the backbone of modern commercially bred cannabis seeds. But these landraces are under threat from habitat loss, government eradication programs, and invasion of foreign varieties. Landrace crop varieties are those that have adapted over time to local environmental conditions, in isolation from other populations of the same species, to a point where they have developed a degree of inbreeding – and typically, a range of unique characteristics (Clarke and Merlin, 2016; McPartland, 2018). This process is generally assisted by some selective breeding and management by humans – but unlike cultivars, landraces are generally more diverse and variable in their expression of traits, and a lot more is left up to natural selection (Salentijn et al., 2015). Landraces may have developed over millennia from ancient lineages that survived in isolation, but may also develop from semi-feral populations of cultivars that have escaped from cultivation sites. *Via* the latter route, it may take just a hundred years or so for a landrace to develop. Landraces are of crucial importance to crop-breeding programs the world over, and have been for centuries. However, modern agriculture depends far too heavily on uniform crop varieties, and due to their encroachment onto vast areas of arable land, biodiversity the world over is threatened. Cultivars are very often bred directly from landraces. In fact, this process is the backbone of commercial cannabis breeding. Cannabis seed breeders have repeatedly drawn from pools of landrace cannabis varieties the world over, and hybridised them to create unique cultivars. The more effort is put into multiple generations of careful crossing and backcrossing, the more stable and true-breeding the final variety will prove to be (Salentijn et al., 2015).

However, when a disease emerges, having a diverse gene pool means there is more chance that individuals with genes for resistance exist among the population. If all individuals are identical, and are susceptible to a particular pathogen, all individuals in the population are equally at risk. As cannabis is often propagated by clones, this is a very real concern for growers the world over.

Given that all these useful traits found in commercial cultivars ultimately derive from landrace parents, it makes a great deal of sense to preserve landrace genepools all over the world. Not only will we therefore preserve buffers against disease, pests and so on – we may also be yet to discover variations and traits that could prove highly useful for development

into new cultivars, for use in medicine, research and industry. The Cannabis genus is now present in every continent save Antarctica, and everywhere it has gone, it has established landraces (Meijer, 1999). The Cannabis genus appears to benefit from a great deal of phenotypic plasticity – this is the quality of exhibiting a great deal of possible variation within a species or genus. Added to this, cannabis has various obvious benefits to humanity, and has been a crop of measurable importance to various world cultures over the millennia of recorded (Small, 2015). Thus, cannabis can express new variations in response to environmental and human pressures, and can do so with relative ease and rapidity (Amaducci et al., 2012).

The main threats to cannabis landrace populations across the world seem to be introduction of foreign genetics, government eradication programs, and habitat loss in general (Small, 2017). Currently, threats to landrace cannabis varieties from climate change are not well evidenced, but in future, this may be another concern to consider. Preservation efforts for traditional landrace varieties (not specific to cannabis) are ongoing, such as those conducted by organizations such as Biodiversity International and the UK's National Institute of Agricultural Botany. As well as this, seed repositories such as the Svalbard Institute are a crucial means of preserving seed varieties in case of future need. Furthermore, there are even some organizations dedicated to preserving cannabis varieties, such as the Vavilov Institute in Russia. Lastly, some cannabis breeders and growers have maintained extensive libraries of landrace genetics, and due to this, certain varieties can be preserved even if they are threatened in the wild. Some landraces are more famous than others, and more in demand. The legendary names from the hippy times are still popular today; the 1970s Manga Rosa, Malawi Gold, Swazi, Limon Verde, Punto Rojo and Colombian Gold are all very special plants, genetics with a real history, a history that lives on in pop-culture, songs, movies, and through word-of-mouth passed on from one generation of stoners to the next.

It is wise to avoid taking foreign varieties, particularly commercial hybrids, to regions that are home to historic landraces (Ranalli et al., 2004; Hilling, 2005; Small, 2015; McPartland, 2018). The preservation and maintenance of landraces depends greatly on their isolation to other populations from the same species, and although the short-term benefits of introducing uniform, high-yielding cultivars may be appealing, the long-term harm to ecosystem and biodiversity may far outweigh any advantages. Landraces could hide cannabis profiles that one day may be used to create new medicines, and these medicines could improve or even save lives. Landraces are also needed to breed and create new strains of cannabis, with new flavors and new effects, which are enjoyed by mankind for medicinal as well as recreational purposes (Small, 2017; Orser, 2019).

There is a growing understanding, both within the communities of patients and scientists that the synergy between multiple components of the cannabis plant is important for the diversity of its therapeutic effects. There are conditions where the effects are mediated by THC or CBD only, but there are many more conditions where these cannabinoids, taken in isolation, have no effect. Cannabis plant

has more than 400 different components, including terpenoids and flavonoids. Most of them have never been investigated, both individually and synergistically. The landraces are a treasure chest, providing the highest variety of different compounds, in contrast to, most commonly, indoor grown strains, selectively bred to enhance THC, and more recently, also CBD content (Chen et. al., 2013; Orser, 2019). This assortment, if researched and investigated properly, can be harnessed and shaped to provide targeted medicines for different illnesses. Landraces have been called "The Holy Grail" of cannabis genetics, preserved and isolated in remote regions around the world.

The formation of cannabis compounds including cannabinoids (THC, CBD, CBN, etc.) and terpenes are heavily affected by the environment a plant is grown in. Things like the soil, weather, and the time of harvest can change the chemical makeup of cannabis. Since landraces are made to grow in the area that they're from, they are considered to have optimal synergistic levels of therapeutic compounds (Ranalli et al., 2004; Small, 2015).

Landraces that fall under the *Cannabis sativa* category originated in Asia and North Africa, but was able to get an early introduction to the West (McPartland, 2018). Later on, they were sent to the Americas, where they tend to thrive in the southern area. These plants are usually taller and have greater internodal lengths than other kinds. Sativa landraces won't be able to mature properly if they are planted at mild northern or southern latitudes (McPartland, 2018; Orser, 2019).

Traced back to the mountains of Afghanistan and India, this landrace (*C. indica*) tends to be shorter, more compact, and more resinous than sativa types. In 1979 when soldiers returned home from conflict in Afghanistan, fresh indica landraces started flowing into Europe and the West. Growers also began forming hybrids by combining indicas with sativas. Indica landraces are ready for harvest early and do well growing in mild, northern latitudes.

Europe, the Himalayas, and Siberia play home to this standout landrace (*C. ruderalis*) that only grows to a few feet tall. Growing in the harsh northern climate, this type isn't potent as it doesn't contain large percentages of THC. Having said that, there is still a purpose for Ruderalis as it's key for breeding, both in marijuana and hemp. The flowering stage is based upon age rather than exposure to light, which sets this kind apart from sativa or indica strains. Days can be as long as twenty hours in the North, so this specific trait is necessary to survive. Breeders use this adaptation, appropriately called „auto-flowering", to produce strains that mature faster (Small, 2015; McPartland, 2018; Orser, 2019).

GENETIC VARIABILITY OF GENUS *CANNABIS*

There is still debate over the taxonomic organization of the genus *Cannabis (Small and Cronquist, 1976; Pollio, 2016; Orser, 2019)*. Some authors have proposed a monotypic genus, *C. sativa*, while others state that two species can be distinguished, *C. sativa* and *C. indica*, and maybe even a third species *C. ruderalis (auto-flowering).* Genetically, the various cannabis varieties are actually very closely related to each other and hard to tease apart (De Meijer and Keizer, 1996; McPartland, 2018). The *C. ruderalis*

originates from the northern parts of Russia and Sibera. In the wild, a pure C. *ruderalis* plant contains next to no THC and produces no psychoactive effects on its own. However these strains have been mixed with other strains because of one unique property. Unlike most varieties of marijuana, *C. ruderalis* or auto-flowering strains will naturally go into flowering on their own, without the need for the 12-12 light change to let the plant „know" it's time to start flowering. They also produce buds sooner than much any other strain of cannabis, and are usually ready for harvest in under 3 months from seed. Originally, *Cannabis ruderalis* was considered a wild breed of cannabis (Small, 2017). However, in recent years it has been brought indoors to influence new hybrid varieties. Modern *C. ruderalis* hybrids usually begin to flower between 21 and 30 days after the seeds have been planted, regardless of the light cycle. This is why most *C. ruderalis* hybrids are attributed as "autoflowering" strains. *C. ruderalis* is a short and stalky plant, especially when compared to its *C. sativa* and *C. indica* counterparts. It typically sits between 1 and 2.5 feet tall at harvest, with a rugged and shaggy growth pattern that produces wide leaflets that express themselves in a light green hue. The buds from the *C. ruderalis* plant tend to be small but still relatively chunky, and are supported by the sturdy, thick stems (McPartland, 2018).

The effects of *Cannabis ruderalis* alone are minimized by its naturally low concentrations of THC. However, the stability and short lifecycle make *C. ruderalis* versatile and attractive to breeders who want to take advantage of its autoflowering trait. *Ruderalis* genes offer the ability for breeders to create an autoflowering hybrid with the advanced potency and flavor profile from its genetic partner. After comparing the genetics of *C. indica, C. sativa*, and *C. ruderalis* it was found that the *ruderalis* gene pool lies somewhere between hemp and drug-type cannabis varieties (Hilling, 2005; Sawler et al., 2015).

Cannabis sativa L. is a stout erect, annual, branching herb varying in size with the climate and soil in which it grows. The extent of branching, like the plant height, depends on environmental and hereditary factors as well as the methods of cultivation. *C. indica* plants are short, densely branched and have wider leaves (Orser, 2019). They are better suited for growing indoors; the species is adapted to its harsh growing environment in countries like Afghanistan, India, Turkey and Morocco and developed some unique characteristics. For example, the plants developed the ability to produce resin — a sticky, organic substance. Resin is very dense in cannabinoids (more on those later) which often makes indica cannabis strains very potent (Meijer, 1999; Hilling, 2005; McPartland, 2018).

C. sativa plants flower later than *C. indica* and the difference in time for seed maturity sufficed for 1 to 2 months (Lynch et al., 2016; Small, 2017). In addition, *C. indica* plants have high THC:CBD ratios while *C. sativa* plants have high CBD:THC ratios. Although morphological differences, both species possess equal THC:CBD ratio (about 200:1). The variation in ratio between these two substances was established in cannabis cultivars. For example, the new distinct cultivar 'Avidekel' (*C. sativa* ssp. *indica*) selected in Israel is characterized by a high amount of Cannabidiol (16.3%) and a very low amount of THC (0.8%) (Friedberg, 2016; Orser, 2019).

One of the easiest ways to tell if a marijuana plant is *indica* or *sativa* is to look at the shape of it's leaves (Small, 2017; Orser, 2019). As with all the categories, hybrids my exhibit either shape, or a combination (Indica – Broad leaf; Sativa – Slender leaf).

The species C. *ruderalis* was first classified in 1924 by the Russian botanist Janischevsky. Janischevsky noticed that this species of cannabis was visibly different than *sativa* and *indica*, growing no higher than two feet tall with a wild, unbranched appearance (Small and Cronquist, 1976). The name *ruderalis* comes from the word 'ruderal', which is a term used by botanists to describe hardy, non-domesticated plants that prefer disturbed soils and environments. Even though *C. ruderalis* can be considered a wild type of cannabis without any of the useful psychoactive or industrial properties of its cultivated cousins, *C. ruderalis* still has some uses for modern breeders because of its particular characteristics (Small, 2017):

- *C. ruderalis* often hassignificant levels of canabidiol and gained popularity with modern breeders. Therefore, they can be crossed with *C. indica* varieties to produce high CBD cultivars for medical use (Pollio, 2016; Friedberg, 2016; Small, 2018).
- *C. ruderalis* plants are auto-flowering. This means that they flower based on maturity, spontaneously going into flower 21-30 days into their growth cycle. They can also continue flowering for the whole season until they are killed by frost (Small, 2018).
- *C. ruderalis* is hardy pest resistance. Therefore, *C. ruderalis*, with its diminutive size, makes for a great addition to the *C. sativa* gene pool. When hybridized, outdoor crops gain protection against pests, and indoor crops can be reduced to a more manageable height. A wide number of molecular markers (RAPD, AFLP) proved the monotypic taxonomy of Cannabis (Datwyler and Weiblen, 2006). RAPD analysis is already accepted as suitable for the identification of the genetic structure and the geographic origin of Cannabis germplasms (Jagadish et al., 1996; Mandolino et al., 2002; Yang et al., 2013). Thus, the genetic study of the famous Italian landrace 'Carmagnola' shows the average polymorphism more than 80%. The inheritance of chemotype was investigated by comparison between the contrasted inbred lines contained only THC or CBD, respectively. The cross between such two parents and analysis of the F_1 generation, and segregation in F_2 allows determining the chemotype-

Leaf shape of *C. sativa*

Leaf shape of *C. indica*

Leaf shape of C. *ruderalis*

associated markers.

Many authors accepted the hypothesis based on geographical origin of cannabis plants thus, determining the geographical regions Cannabis was likely to have evolved. For example: Northern (Northern Russian, Finland), Central ecotypes (Central Russian, Ukraine), Southern ecotypes (Mediterranean region, Balkan, Turkey, Caucasus), and Far Eastern ecotypes (China, Japan and Korea).

One of the biggest collections of Cannabis is located in gene bank of the Vavilov Research Institute (VIR), Russia (about 200 samples); the gene bank of Hungary maintained about 70 samples, and collection with more than 20 samples have Germany, Japan and Turkey. Unlike the other culture plants, the available samples of Cannabis germplasms are insufficiently described.

BREEDING OF CANNABIS

Hemp is open pollinated (wind-pollinated) and is usually a dioecious annual crop, where female and male flowers are on different individuals, indicating that hemp is naturally outcrossing (cross-pollinator). All cannabis strains can intercross creating, in some cases, a continuous pattern of variation (Salentijn et al., 2015). In hemp the control of pollination is therefore an important issue. In the case of a dioecious hemp population the male and female plants are intermixed and the female plants are always cross-pollinated (Small, 2015). Entirely female populations exist that can be used to produce hybrid cultivars by crossing with a selected pollen donor. In the case of monoecious hemp cultivars the male and female flowers are on the same individual, which enables selfing. The breeding of a cross-pollinator such as hemp requires a specific breeding approach that comprises three breeding phases (Ranalli, 2004; Posselt, 2010): (1) search for the natural variation in the material and create a base population, (2) generate varietal parents through selection and improve the population through recurrent selection steps to create a breeding population, and (3) develop and test experimental cultivars (Canapasemi, 1988; Salentijn et al., 2015).

The hemp cultivars available are mainly population cultivars, such as 'open pollinated cultivars' that are the result of recurrent selection and 'synthetic cultivars' that are advanced generations of a population initiated by crosses among a restricted number of selected parents and multiplied by a number of random out-crossings in isolation (Ram and Set, 1982; Salentijn et al., 2015).

The methods commonly used in hemp breeding are 'mass selection', 'cross-breeding', 'inbreeding', and 'hybrid breeding', and more recently there are a few examples of the use of molecular markers to assisted breeding.

Traits that are important in cannabis breeding comprise: high fiber yield and fiber quality, cannabinoid content and composition, degree of monoecy, length of vegetative cycle, and resistance to diseases and pests. A great deal of attention is given to high fiber yield and quality together with low THC content (Canapasemi, 1988; Amaducci et al., 2000). Some traits show a high plasticity, especially cannabinoid content and phenological development. Because hemp is very sensitive to environmental conditions, such as day length and temperature, cultivars are typically developed for specific environments and cropping

conditions (Salentijn et al., 2015).

Given the strong influence of the environment on hemp biomass yield and quality, hemp cultivars were developed for specific environments and end-uses. As result of the efforts in hemp breeding specific cultivars were designed for cultivation in Italy, France, Hungary, Poland, Romania, Russia (former USSR), former Yugoslavia, Spain, former Czechoslovakia, Germany, The Netherlands, Finland, Canada, and China. For example, Ukrainian cultivars and French cultivars differ in the length of their vegetative period (Salentijn et al., 2015). For seed production, flowering, and seed ripening is required. Therefore early ripening cultivars are more suitable for seed production in Northern Europe, where the growing season is short, and late ripening cultivars are suitable for the same use in the South of Europe. As the fiber formation finishes already a month before ripening, late cultivars are often grown in Northern regions, for the production of stem and high quality fibers. In the Northern regions late flowering cultivars have a prolonged vegetative phase and a higher stem yield, in situations where flowering and seed ripening is not required. The harvest can be performed at different developmental stages depending on the use.

The recent increasing demand in the market for cannabinoid-CBD will result in a rapid growth of production for medicine-type hemp (high CBD content but less than 0.2% THC in Europe Union or 0.3% in other countries). Thus new cultivars need to be developed.

Breeding for low delta-9-tetrahydrocannabidol (THC) has been a main target in fiber hemp breeding and levels below <0.2% THC have been reached for some cultivars. Government regulations that allowed a THC content of only 0.2% were implemented in the European Union in 2001. Since then, a further and stable reduction of THC gained importance as a breeding goal. In the former USSR a successful breeding program for the reduction of cannabinoids was initiated in the 1970s. Cultivars completely lacking THC were obtained. In a joint effort between scientists in France and Ukraine several new monoecious cultivars were developed. These cultivars have very low THC levels (THC < 0.07%) and lack the typical hemp aroma (e.g. USO-45). The methods commonly used in hemp breeding are 'mass selection', 'cross-breeding', 'inbreeding', and 'hybrid breeding' (De Meijer and van Soest, 1992).

The historical importance of hemp cultivation in Europe is well reflected by the abundance of cultivars, traditional landraces, and populations that were selected in the main areas of hemp cultivation throughout Europe. Mass selection was used in the past to select the most important cultivars, such as Carmagnola in Italy or Novosasdka konoplia in Yugoslavia (Ranalli, 2004; Posselt, 2010). In mass selection pollination cannot be controlled and any improvement in fiber content is very slow. A large contribution to the increase of stem fiber content was obtained by the application of the Bredemann method that consisted in the individual selection of male plants on the basis of the fiber content, measured on a longitudinal section of the stem (Salentijn et al., 2015; Rahn et al., 2016).

Canada has become the main supplier of hemp seed and oil-cake for the United States. Field production was dominated by the cultivars Finola (originating from Finland), Crag (Canada), and USO 14 (Ukrainian) for a long time. Several

breeding programs included developing commercial strains from feral Canadian stocks, creating superior oilseed cultivars by increasing seed yield and optimizing fiber use for a variety of regions.

The term sinsemilla refers to a cultivation technique rather than a genetic strain. *Cannabis* with the highest level of THC is comprised exclusively of the female flower heads ("buds") that remain unfertilized throughout maturity and which, consequently, contain no seeds (Small, 2017). The production of sinsemilla requires identifying the female plants and ensuring that they are not exposed to pollen.

The first and most obvious boost to sinsemilla production was the use of clones. Cloning simply means propagating from a successful "mother" plant. This cutting is rooted and transplanted. It is a genetic duplicate of its mother and thus can be used to create even more cuttings. A square metre of mother plants can provide numerous clones per week.

Cannabinoid biosynthesis requires phenol and terpenoid precursors (Lynch et al., 2016). Cannabinoid content differs in terms of quantity and quality. Cannabinoid quantity (dry weight percentage) is affected by many genes (polygenic), and modulated by environment (Sawler et al., 2015). Cannabinoid quality (the cannabinoid profile or chemotype) is largely genetic-possibly monogenic. Gene's determine a plants chemotype and the expression of cannabinoid-producing machinery (density of capitate stalked glandular trichomes, size of resin heads). Gender is another genetic factor; female flowers produce more canabinoids than male flowers. Environmental factors include photoperiod, light quantity and quality, soil nutrients, and temperature. Valid quantitative comparisons between plants must minimalize environmental variables (Salentijn et al., 2015; Small, 2017).

The distinction between fiber and drug accessions can only be made on the basis of the cannabinoid profile (chemotype) (Lynch et al., 2017). Three major 'chemotypes' are recognized in hemp based on the ratio in the inflorescence dry matter between the two major cannabinoids of hemp, delta-9-tetrahydrocannabidol (THC) and cannabidiol (CBD):

(i) drugs type with THC prevalent, (THC > 0.3% и CBD < 0.5%);

(ii) intermediate type with similar amounts of both THC and CBD;

(iii) fiber type with CBD prevalent. Two alleles at the B locus (B_T and B_D) are controlling the trait. The other chemotype are minor chemotypes that are not frequently found.

Cannabis chemotypes having no cannabinoids, or only CBG (cannabigerol) or CBC (cannabichromene) are interesting because of their pharmaceutical value. CBC dominates the cannabinoid fraction of juvenile cannabis plants and declines with maturation.

It was established that the plant chemotype depends on the geographic origin but many authors have reported that cannabinoid profile of the plants is under strict genetic control. According to Beutler and Manderosian (1978), the CBD/THC ratio is a chemical marker with taxonomic value. Molecular markers for analyses the chemotype could be used for identification the THC producing plants (Beutler and Manderosian, 1998; de Meijer et al., 2003).

INDOOR PRODUCTION

The main production of cannabis worldwide is still outdoors and these plants are generally but not necessarily grown from seeds. Cannabis with the highest level of THC is comprised exclusively of the female flower heads ("buds") that remain unfertilized throughout maturity and which, consequently, contain no seeds (Cervantes, 2006; Small, 2017). Growing cannabis from seed means that half of the crop might be unwanted male plants. For cost-intensive greenhouse production this is usually avoided, which can be achieved easily by cloning. Cloning and indoor production go hand in hand. Indoor production is mainly encountered in technologically advanced countries, where big basements or closed factories are usually used (Cervantes, 2006; Bouchard, 2008).

Asexual propagation (cloning) allows the preservation of genotype because only normal cell division (mitosis) occurs during growth and regeneration. The vegetative (non-reproductive) tissue of *Cannabis* has 10 pairs of chromosomes in the nucleus of each cell. This is known as the diploid (2n) condition where 2n = 20 chromosomes. During mitosis every chromosome pair replicates and one of the two identical sets of chromosome pairs migrates to each daughter cell, which now has a genotype identical to the mother cell. Consequently, every vegetative cell in a *Cannabis* plant has the same genotype and a plant resulting from asexual propagation will have the same genotype as the mother plant and will, for all practical purposes, develop identically under the same environmental conditions (Hilling, 2005; Small, 2017).

In *Cannabis*, mitosis takes place in the shoot apex (meristem), root tip meristems, and the meristematic cam- bium layer of the stalk. A propagator makes use of these meristematic areas to produce clones that will grow and be multiplied. Asexual propagation techniques such as cuttage, layerage, and division of roots can ensure identical populations as large as the growth and development of the parental material will permit. Clones can be produced from even a single cell, because every cell of the plant possesses the genetic information necessary to regenerate a complete plant. Asexual propagation produces clones which perpetuate the unique characteristics of the parent plant. Because of the heterozygous nature of *Cannabis*, valuable traits may be lost by sexual propagation that can be preserved and multiplied by cloning (Campbell et al., 2019). Propagation of nearly identical populations of all-pistillate, fast growing, evenly maturing *Cannabis* is made possible through cloning. Any agricultural or environmental influences will affect all the members of that clone equally.

The concept of clone does not mean that all members of the clone will necessarily appear identical in all characteristics. The phenotype that we observe in an individual is influenced by its surroundings (Campbell et al., 2019). Therefore, members of the clone will develop differently under varying environmental conditions. These influences do not affect genotype and therefore are not permanent. Cloning theoretically can preserve a genotype forever. Vigor may slowly decline due to poor selection of clone material or the constant pressure of disease or environmental stress, but this trend will reverse if the pressures are removed. Shifts in genetic composition occasionally occur during selection for vigorous growth. However, if parental strains are maintained

by infrequent cloning this is less likely. Only mutation of a gene in a vegetative cell that then divides and passes on the mutated gene will permanently affect the genotype of the clone. If this mutated portion is cloned or reproduced sexually, the mutant genotype will be further replicated.

Industrial cannabis (industrial hemp) comprises a number of varieties of *Cannabis sativa* L. that are intended for agricultural and industrial purposes (Canapasemi, 1988; Small, 2017). They are grown for their seeds and fibres. Industrial cannabis is characterized by low THC content and high cannabidiol (CBD) content. In most European countries the current upperlegal limit for cultivation is 0.2 per cent THC (Canada: 0.3 per cent). Harvesting for fibres occurs at the end of flowering of the female plants and before seed formation (Hillling , 2005; Chen et al., 2013; Clarke and Merlin, 2016; Lynch et al., 2016).

The THC content varies depending on the plant part:

- 10-12 per cent in pistillate flowers;
- 1-2 per cent in leaves;
- 0.1-0.3 per cent in stalks;
- < 0.03 per cent in the roots.

It is still the traditional belief that only the fruiting and flowering tops and leaves next to the flowering tops contain significant quantities of the psychoactive constituent (THC); they are known as the "drug-containing parts", and generally it is only these parts of the plant that are sold in the illicit market. Indeed, these parts contain the highest amount of THC. However, illicitly consumed herbal cannabis also includes bigger leaves located at greater distance from the flowering tops. The dried leaves and flowers of the cannabis plant are known as "marihuana", and a plethora of other regional names exist. "Marihuana" is found in the illegal market unchanged, i.e. raw from the plant (also called "dried flower"), processed as compressed slabs or coins, or as ground up material. The presentation of the herbal material in illicit markets varies widely, from region to region as well as within the countries of each region (Small, 2017).

Cannabis sativa contains a unique class of terpeno-phenolic compounds (cannabinoids or phytocannabinoids) which have been extensively studied since the discovery of the chemical structure of tetrahidrocannabinol (Δ9-THC) commonly known as THC, which is the main constituent responsible for the psychoactive effects (Hussein, 2014; Lynch et al., 2016). The accumulation of THC is mainly found in glandular trichomes of the flowers of the female plant. A total of 537 Cannabis constituents including 109 phytocannabinoids have been reported in *C. sativa*. Pharmaceutical research companies are developing new natural cannabinoid formulations and delivery systems that will meet government regulatory requirements. Cannabis is used to relieve nausea and secondary to chemotherapy, pain, vomiting, spasticity in multiple sclerosis and increase hunger in anorexia (Salentijn et al., 2015).

IN VITRO CELL CULTURE OF *CANNABIS* TO PRODUCE CANABINOIDS

The culturing of plant cells *in vitro* is the first step in a number of specialized agri-industrial and biotechnological processes. Living cultures can be use for micro-propagation, haploid production, somatic embryo-genesis, synthetic seed production

callus cell production, protoplast isolation and much more (Hussein, 2014).

Large scale agriculture is at its most efficient when it has a reliable, uniform supply of juvenile plantlets to grow from. Cannabis culture is no different, and starting vegetation phase of growth from already hardened plantlets (as opposed to seeds) saves time and resources otherwise spent on attempting to propagate non-viable or substandard / undesirable seeds within a group. An additional advantage in regulated environments is that the entirety of permitted canopy space can be devoted to growing plants instead of maintaining vegetative mothers and clones. Clonal (that is, genetically identical) plantlets – derived either from mother plant cuttings, or other methods– add the even more important advantage of maintaining uniform strain genetics, leading to predictable growth behaviour and mature plant characteristics such as THC and CBD content, terpene profiles, and other strain specific traits which are associated with a strain name and any downstream product branding (Hussein, 2014).

Tissue culture from plants involves taking a small amount of plant tissue which is induced to return to- and maintained in- a primitive stage (in the form of 'calluses'). These cells can be propagated indefinitely in defined synthetic media; when plantlets are desired, some of these cells are taken out and then induced to differentiate into all of the different tissue that makes up a complete plant. The first benefit from the method is that these cells can be maintained in large quantities in very small volumes and can be expanded to hundreds of thousands of cells very quickly, and again, in very small volumes (Wang et al., 2009; Wrobel et al., 2018). The second benefit is in scalability; hundreds of thousands of plantlets can be induced in far less time and effort than creating mere hundreds of cuttings (Helmphill et al., 1978). A crucial third benefit of tissue culture over traditional cloning methods is that by stringent control of the maintenance conditions. For example, appropriate growth media additives, and proper handling techniques combined with good facility sanitation practices, strains can be maintained and propagated free of detectable pathogens (tissue culture methods require aseptic handling and the presence of pathogens are readily detected and eliminated). So, it was appreciated that tissue culture can generate vast numbers of identical, healthy "calluses"; how do we go about turning these into plants that we recognize as plants, suitable to move forward to vegetation and flowering stages?

The process generally involves taking callus stock, expanding the number of cells in liquid media as single cells, then plating individual cells in their own culture chambers, then add the appropriate growth factors and hormones that induce them to differentiate into all of the normal tissue types that make up complete plants; as the cells divide they will differentiate into roots, stems, and leaves (Wang et al., 2009; Wrobel et al., 2018). Once this differentiation proceeds, they grow into complete plantlets. When these plantlets reach around the 6-8" tall stage, these are then ready to pass on for handling as normal juvenile plants, without any differences in process for subsequent growth.

An added benefit of tissue culture processes is that calluses can be maintained long term without differentiation, at very low cost and low space requirements. Particularly for an operation wanting

to maintain multiple strain types for long periods, but able to bring them into production quickly and in large numbers, tissue culture is an economical and reliable solution (Wrobel et al., 2018).

Cell culture is a well-established method for the propagation of a vast numbers of healthy, uniform juvenile plants; therefore, it is quite reasonable to apply this approach in cannabis production (plant breeding, secondary metabolites biosynthesis, etc.). This method represents a good model to overcome many problems linked to the conventional agriculture such as variations in the crop quality due to environmental factors: drought, flooding and other abiotic stresses and/or biotic stresses as diseases or pest attacks (Helmphill et al., 1978; Wang et al., 2009). Moreover crop adulteration, losses in storage and handling may decline the secondary metabolites production, which cannot be prevented by inability of some authorities. Unfortunately, until now, canabinoids were not found in cell suspensions or callus cultures. There are some technologies aiming to influence the canabinoids synthesis, for example, using of different explant types, modification of nutrient conditions etc.

- **Explants** – it is a technique used for the isolation of cells from a piece or pieces of tissue. Tissue harvested in this manner is called an explant. It can be a portion of the shoot, leaves, flowers or some cells from a plant. The tissue is harvested in an aseptic manner, often minced, and pieces placed in a cell culture dish containing growth media. Over time, progenitor cells migrate out of the tissue onto the surface of the dish. These primary cells can then be further expanded and transferred into fresh dishes. In the case of cannabis, it was suggested to use the flowers of the female plant to make explants, as it is in those flowers where the cannabinoids are produced in the plant.
- **Callus** – the first step for obtaining cell culture is inducing callus. This stage involves the dedifferentiation of plant material. Differentiated cells that are part of the plant tissues will lead to an unorganized group of cells that are called callus. These cells can regenerate a new plant, this ability is called totipotency (describes the ability to regulate cell division and give rise to all different cell types of the vegetable body). But for this change of tissue, it is essential a certain combination of growth regulators or phytohormones. In our case, growth regulators are always added to the culture medium of Murashige-Skoog (MS) medium used previously by other authors, for the in vitro culture of the plant species Sterile explants are cut in pieces of about (0.2 x 0.2) cm^2. They are then transferred into petri dishes with solid MS medium. The medium brings the auxin responsible of the callus induction 2,4-D at 1 mg/L.
- **Cell suspension** – callus obtained in the petri dishes are used to set up cell suspension cultures. Approximately 1.5 g of callus from each explant is chopped to smaller particles and suspended into 250 mL flask with 50 mL of liquid MS medium. The suspension cultures are grown at 25ºC under continuous light (intensity 1,200-1,800 lux) on a gyratory shaker at 110 rpm. After 2 weeks, the contents of each flask is maintained in B5 medium (B5 components, 2,4-D 2.0 mg/L, IAA 0.5 mg/L, NAA

0.5 mg/L, K 0.2 mg/L and sucrose 30 g/L). These cell cultures are then maintained by sub-culturing weekly.

- **Elicitation** – Plants or plant cells *in vitro*, show physiological and morphological response to microbial, physical or chemical factors which are known as 'elicitors'. Elicitation is a process of induced or enhanced synthesis of secondary metabolites by the plants to ensure their survival persistence and competitiveness. The application of elicitors, which is currently the focus of research, has been considered as one of the most effective methods to improve the synthesis of secondary metabolites in medicinal plants. Plant secondary metabolites are unique sources for pharmaceuticals, food additives, flavours and other industrial materials. Accumulation of such metabolites often occurs in plants subjected to stresses including various elicitors or signal molecules. Commonly tested chemical elicitors are salicylic acid, methyl salicylate, bezoic acid, chitosan and so forth which affect production of phenolic compounds and activation of various defense-related enzymes in plants. Plants are challenged by a variety of biotic stresses like fungal, bacterial or viral infections. This lead to the great loss to a plant yield.

Elicitors can be divided into two types on the basis of nature, biotic and abiotic. Biotic elicitors are the substances of biological origin, which includes polysaccharides originated from plant cell walls (chitin, pectin, cellulose, etc.) and micro-organisms. Abiotic elicitors consist of the substances that are of non-biological origin and are grouped into physical, chemical and hormonal factor.

Abiotic elicitors have wide range of effects on the plants and in the production of secondary metabolites. The use of abiotic elicitors in plant cell cultures has received less attention compared with the biotic elicitors. Recent research works explained the functions of many key genes, proteins, metabolites and molecular networks involved in plant responses to heavy metals, light, drought, salinity, thermal, hormonal and other abiotic elicitors.

Chemical Elicitors: Metals like Ni, Ag, Fe and Co have been shown to elicit the production of secondary metabolites in a number of plants. Many kinds of heavy metal were also used as elicitors to induce accumulations of bioactive compounds, such as Co^{2+}, Ag^{+}, Cd^{2+}, Cu^{2+}, Ce^{3+}, La, Mn^{2+} and Zn^{2+}.

Physical Elicitors: Ultrasound, light, osmotic stress, salinity, drought and thermal stress are some of the physical elicitors.

Hormonal Elicitors: Salicylic Acid – the one of the important abiotic elicitor, which has the capability to induce the secondary metabolites from in vitro cultures; **Jasmonates** – Jasmonic acid has been proposed as key compound of the signal transduction pathway involved in the elicitation of secondary metabolite biosynthesis which takes part in plant defense reactions.

The response to elicitors can vary depending on various factors including the specificity of elicitors interval addition, the culture conditions or the concentration of the elicitor. This last factor can affect the intensity of the response, and each may have a different plant at the same concentration of elicitor response, which causes only the effective dose can find empirically.

Cannabinoids have not been detected in cell suspension or callus cultures induced from cell suspension cultures. Some of the strategies used to stimulate cannabinoid production from cell cultures involved media modifications and a variety of explants. For elicitation, dry mycelium suspensions of two fungal strains *Pythium aphanidermatum* (Edson) Fitzp. and *Botrytis cinerea* Pers. (isolated from cannabis plants) were used. In addition, it was suggested that biotic elicitors such as chitosan, chitin and elicitins, and abiotic elicitors such as sodium orthovanadate и vanadyl sulphate employed in different concentrations would be able to induce secondary metabolite production in *C. sativa* cell cultures. We propose chitosan, chitin and elicitins that are biotic elicitors and Sodium orthovanadate and vanadyl sulphate as chemical abiotic elicitors, from here we would have to experiment with these elicitors at different concentrations to test if the cannabinoid biosynthetic pathway is activated.

CANNABINOIDS BIOSYNTHESIS

Conversion of CBD acid to THC acid is the single most important reaction with respect to psychoactivity in the entire pathway (Fellenmeier et al., 2001; Siricantarama and Taura, 2017). Actually, THC acid and the other necessary cannabinoid acids are not psychoactive until they decarboxylate (lose an acidic carboxyl group [COOHI). It is the cannabinoid acids which move along the biosynthetic pathway, and these acids undergo the strategic reactions that determine the position of any particular cannabinoid molecule along the pathway. After the resins are secreted by the glandular trichome they begin to harden and the cannabinoid acids begin to decarboxylate. Any remaining cannabinoid acids are decarboxylated by heat within a few days after harvesting. Other THC acids with shorter side-chains also occur in certain trains of *Cannabis*. Several are known to be psychoactive and many more are suspected of psychoactivity (Andre et al., 2016). The shorter propyl (three- carbon) and methyl (one-carbon) side-chain homologs (similarly shaped molecules) are shorter acting than pentyl (five-carbon) THCs and may account for some of the quick, flashy effects noted by some marijuana users.

CONTROL OF GENDER (MALE FLOWERING CONTROL)

Monoecious hemp cultivars have a higher seed yield and higher uniformity compared to dioecious cultivars and therefore mechanical harvesting of such cultivars is easier. Drawbacks are the narrower genetic base, necessity to maintain the monoecious trait (including the selfing of a monoecious plant and elimination of male plants), strict isolation of propagations and seed batch control for male plants (Hilling, 2005; Small, 2017). In dioecious cultivars, selection of males before pollination and pollination only with the best-scoring male is a common practice in breeding (Posselt, 2010; Small, 2017).

The determination of the gender in *C. sativa* L. is influenced by both genetic and environmental factors. In cannabis, two sex chromosomes, X and Y are present, whereby the Y chromosome is much larger than X chromosomes and autosomes (Moliterni et al., 2004; Calakos et al., 2017). True male plants have one X and one Y chromosome, females have two

X chromosomes resulting in a difference in genome size between male and female plants as determined by flow cytometry.

However, as is the case in many other plant species, in *C. sativa* L., determination of gender is not only controlled by sex-chromosomes and can be altered by chemicals such as growth regulating hormones or silver thiosulfate. Gibberellic acid induces male characters while auxins, ethylene and cytokines induce femaleness, silver thiosulfate promotes male flowers on female (XX) plants and is a useful tool for producing seeds that give rise to only female plants. In dioecious hemp cultivars differences in growth rate and development between male and female plants are evident whereby the male plants tend to flower and_senesce earlier. As such, compounds that control gender are useful tools to achieve uni-sexuality and uniformity in hemp (Small, 2017)

Several markers for maleness have been identified (Jagadish et al., 1996; Alghanim et al., 2003; Datwyler and Weiblen, 2006; Adhikari et al., 2016). SCAR markers fragments were present in both female, male and monoecious plants but a single band was specific for male plants. These are thus not the primers themselves but feature in the region amplified that is male specific. A specific marker (SCAR, OPA08; developed on the MADC2 region; 391 bp fragment in male plants and two larger fragments in females and monoecious plants) allows the safe identification of male plants in dioecious and monoecious cultivars at all developmental stages with a quick and easy, direct PCR method. A marker for the monoecious trait is still required to fully characterize the sexuality in hemp. Obstacles are the environmental influences altering the expression of male flowers in monoecious plants that can change the female:monoecious rate.

FLOWERING AND HARVESTING

Flowering usually starts when darkness exceeds eleven hours per day (Pollio, 2016; Small, 2017). The flowering cycle can last anywhere between four and twelve weeks, depending on the strain and environmental conditions. Flowering times given by seed companies usually refer to the time taken to flower when grown from seed. Plants grown from cuttings can take a week or so longer to finish flowering.

A good sign of ripeness is the colour of the hair-like structures (stigmas). As each flower ripens, these usually shrivel and turn brown. When about 75 per cent of the stigmas are brown, the plants are ready to harvest (Cervantes, 2006; Small, 2017).

LIFE CYCLE OF *CANNABIS*

- **Seed germination** – nearly every cultivated *Cannabis* plant, no matter what its future, began as a germinating seed; and nearly all Cannabis cultivators, no matter what their intention, start with seeds that are gifts from a fellow cultivator or ex- tracted from imported shipments of marijuana. Seeds are planted in the spring and usually germinate in 3 to 7 days. The seedling emerges from the ground by the straightening of the hypocotyl (embryonic stem). The cotyledons (seed leaves) are slightly unequal in size, narrowed to the base and rounded or blunt to the tip. The hypocotyl ranges from 1 to 10 centimeters in length. Under favorable

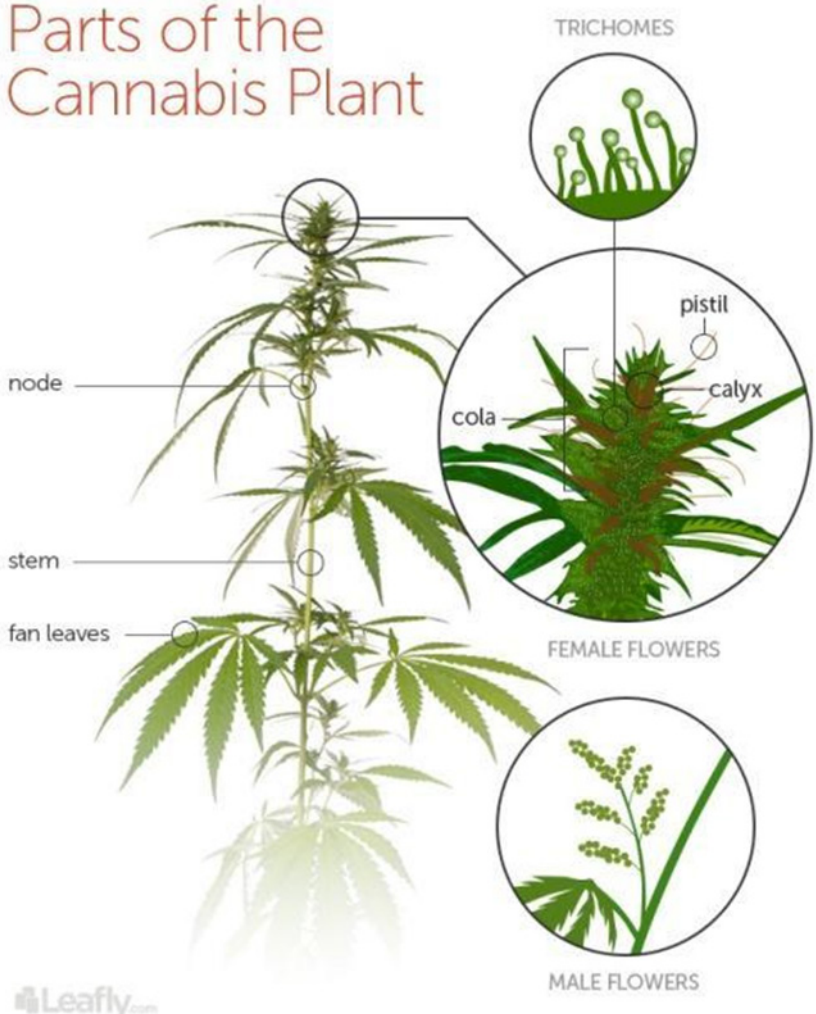
conditions *Cannabis* grows up to 7 centimeters a day in height during the long days of summer. The medicinal value of seed germination is quite limited because of the insignificant synthesis of canabinoids.

- **Juvenile (Seedling) Stage** – the cannabis plant is receiving light through its embryonic leaves, it will focus its energy into producing a more substantial foliage. It starts to produce 3 fingered leaves, making it start to look more like a cannabis plant. As the seedling continues to grow, more and more of these leaves will start to form. The seedling stage often lasts 1 to 3 weeks; it is done when the cannabis plant has created 4 to 8 new leaves during this time. At this stage is still difficult to determine the gender of a plant but DNA from male plants was identified by RAPD markers. During this stage, the medicinal value of the plants is still low.
- **Vegetative stage –** one of the major phases of cannabis growth. This is the stage where the plants really flesh out, and focus on maximum growth before they flower. During this time of their lives, cannabis plants will require a lot of energy, and need a lot of light and nutrition to produce it. It is during this stage that the plant really takes on the stereotypical form of a cannabis plant, and will grow rapidly in both height and width, producing a thicker stem with many more branches and fully fingered leaves. This growth will maximize the plant's ability to produce flowers with its increased surface area for light exposure, and structural integrity - which is, at the end of the day, the reason for growing cannabis. The sex of the cannabis will also begin to be distinguishable towards the end of this stage, allowing for the male plants to be removed from the grow area before they have the chance to release their pollen. The whole vegetative stage will usually take anywhere between 1 to 5 months to complete. During this stage, the biosynthesis of cannabinoids is initiated.
- **Flowering stage** – Flowering usually starts when darkness exceeds eleven hours per day. The flowering cycle can last anywhere between four and twelve weeks, depending on the strain and environmental conditions. Many factors contribute to determining the sexuality of a flowering *Cannabis* plant. Under average conditions with a normal inductive photoperiod, *Cannabis* will bloom and produce approximately equal numbers of pure staminate and pure pistillate plants with a few hermaphrodites (both sexes on the same plant). Under conditions of extreme stress, such as nutrient excess or deficiency, mutilation, and altered light cycles, populations have been shown to depart greatly from the expected one-to-one staminate to pistillate ratio. The differences in flowering patterns of male and female plants are expressed in many ways. Soon after dehiscence (pollen shedding) the staminate plant dies, while the pistillate plant may mature up to five months after viable flowers are formed if little or no fertilization occurs. Compared with pistillate plants, staminate plants show a more rapid increase in height and a more rapid decrease in leaf size to the

bracts which accompany the flowers. Staminate plants tend to flower up to one month earlier than pistillate plants; also, the staminate plants die after shedding pollen. After approximately 14 to 35 days the seed is matured and drops from the plant, leaving the dry calyx attached to the stem. This completes the normally 4 to 6 month life cycle, which may take as little as 2 months or as long as 10 months. Fresh seeds approach 100% viability, but this decreases with age. The hard mature seed is partially surrounded by the calyx and is variously patterned in grey, brown, or black.

USE OF COLCHICINE TO PRODUCE POLYPLOID PLANTS OF CANNABIS

Polyploid cells and organisms are those containing more than two paired (homologous) sets of chromosomes. Most species whose cells have nuclei (Eukaryotes) are diploid, meaning they have two sets of chromosomes-one set inherited from each parent. Polyploidization is widespread in plants, and is an important mechanism of speciation. Polyploids can be formed in various ways. The study of polyploids has both important theoretical significance



and valuable applications. The production and application of polyploidy breeding have brought remarkable economic and social benefits (Parsons et al., 2019).

Polyploidy (favorable traits in Cannabis) has not been shown to occur naturally in Cannabis; however, it may be induced artificially with colchicine treatments. Colchicine is a poisonous compound extracted from the roots of certain Colchicum species; it inhibits chromosome segregation to daughter cells and cell wall formation, resulting in larger than average daughter cells with multiple chromosome sets.

There are several studies on the efficiency of colchicine on inducing polyploidy in *Cannabis sativa* and investigation of effects of polyploidy induction on some primary and secondary metabolites (Bagheri and Mansouri, 2015; Parsons et al., 2019). Shoot tips and seeds were treated with different concentrations of colchicine and time through dropping method. The ploidy levels were screened with flow cytometry. The biggest proportion of the tetraploids (43.33%) and mixoploids (13.33%) was obtained from the 24-h treatment in 0.2 and 0.1% w/v, respectively. Colchicine with 0.2 % concentration and 48 h duration was more destructive than 24 h. The biochemical analyses showed that reducing sugars, soluble sugars, total protein, and total flavonoids increased significantly in mixoploid plants compared with tetraploid and diploid plants. Tetraploid plants had a higher amount of total proteins, total flavonoids, and starch in comparison with control plants. The results showed that polyploidization could increase the contents of tetrahydrocannabinol in mixoploid plants only, but tetraploid plants had lower amounts of this substance in comparison with diploids. Also, it was found such changes in protein concentration in electrophoresis analysis. In overall, the studies suggested that tetraploidization could not be useful to produce tetrahydrocannabinol for commercial use, and in this case, mixoploids are more suitable.

It was reported that the height of tetraploid (4n) Cannabis often exceeded the height of the original diploid plants by 25-30%. Tetraploids were intensely colored, with dark green leaves and stems and a well-developed gross phenotype. Increased height and vigorous growth, as a rule, vanish in subsequent generations. Tetraploid plants often revert to the diploid condition, making it difficult to support tetraploid populations.

CANNABIS-BASED MEDICINE

Cannabis is still sending “signals of misunderstanding”. The result is an exaggeration of beneficial or deleterious effects as well as occasional intermixture of medical science with other moral categories (Friedberg, 2016). The pharmacological classification of cannabis is controversial. It has been characterized as a sedative-hypnotic-general anesthetic like alcohol and nitrous oxide; a mixed stimulant-depressant; a mild hallucinogen, especially at higher doses; a “psychedelic”, like LSD at very high doses; and as a separate category of psychic experience. All these terms are problematical. None of them is completely satisfactory to denote the euphoric psychological effects of marijuana in general and THC in particular.

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