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Cannabis sativa: Polyploidization - Triploid and Tetraploid Production

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Abstract

Cannabis sativa is known for the source of therapeutic compounds, gaining great importance since restrictions on its growth and use are gradually reduced throughout the world. Unintended cross-pollination of *Cannabis sativa* crops is one of the most important threats to cannabinoid production. Therefore, avoiding pollination of female flowers during the production of *Cannabis sativa* for phytocannabinoids is a priority for growers of this crop, as pollination has been shown to reduce final yield of phytocannabinoids. Polyploid plants possess three or more sets of homologous chromosomes and are sterile. Ploidy manipulation has been used in other crops to improve agronomic traits, reduce fertility and produce sterile plants. A promising approach for cannabis breeding involves the process of polyploidization, in which the number of copies of each chromosome is increased. Polyploidization not only causes seedlessness but also brings other physiological and morphological changes to plants, including the plant architecture. Triploid plants are usually both male and female sterile and seeds are not viable or not present. Seedlessness is a highly desirable characteristic for consumers. Polyploid plants often exhibit altered traits, such as larger cell sizes, increased biomass, and changed morphology, which can have significant implications for cannabis industries. Polyploid cannabis plants offer a host of benefits, and they may help growers to overcome the fertilization issue when it comes to cultivating cannabis on a large scale. The strong polyploidy inducing agents in plants are either chemical (Colchicine, Oryzalin, Caffeine, Trifuralin, or phosphoric amides) or gaseous i.e. Nitrous oxide. Polyploid cannabis could become another option for commercial and small-scale growers looking to produce seedless weed. The research lays important groundwork for the development of improved cannabis strains and novel germplasm for breeding efforts.

Keywords: Breeding; Cross Pollination; Cannabis; Diploid; Drug Type; Oryzalin; Polyploidy; Triploid; Tetraploid; Sterile Plants

1. Introduction

Cannabis sativa L. belongs to the family *Cannabaceae* is a dioecious plant, producing male and female flowers on separate unisexual individuals, a trait regulated by an XY chromosome sex determination system [1-37-45]. *Cannabis sativa* L. is a wind-pollinated, although monoecious plants (male and female flowers on same plant) can occur in some population or developed by breeding efforts[1-37-45]. The recent wave of cannabis legalization in various regions has spurred considerable scientific interest in optimizing cultivation techniques, developing new cultivars, and exploring potential medical applications[158]. In commercial production, medical cannabis (drug or marijuana type) plants are all genetically female and male plants are destroyed as seed formation reduces flower quality [1-37]. Male plants die shortly after flowering[1-37, 46]. The female plants live 3 to 5 weeks until seed is fully riped [1-37-45]. Therefore, the plants are obligatory out-crossers [1-37, 46]. While for Industrial *Cannabis sativa* (grain or fibre) production, both male

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and female plants are grown and valued, but only female plants are desired for the medicinal and recreational cultivation of cannabinoids, which are produced at high concentrations in the glandular trichomes located on the bracts of female flowers [1-37-45, 46]. Avoiding pollination of female flowers during the production of *Cannabis sativa* for cannabinoids is a priority for growers of this crop, as pollination has been shown to reduce essential oil yield by more than 55% [1-45, 46]. Furthermore, in the trimmed flower market, the presence of seeds is undesirable for the consumer [1-45, 46]. Growing only genetically female plants is the most common strategy currently used to avoid accidental pollination [1-45, 46]. Although molecular tests are available to identify genetic males prior to transplanting in the field, using either "feminized seed" (seeds that are produced using two genetic females) or vegetatively propagated females are the current industry standards [1-45, 46]. Despite the ease with which genetic females can be produced, the occasional production of male flowers on genetically female *Cannabis sativa* plants (referred to as "hermaphrodism") still necessitates scouting of fields and the removal of pollen-bearing plants for successful, essential oil hemp production[1-45, 46].

Pollen drift from outside a managed field presents another challenge for *Cannabis sativa* growers[46]. *Cannabis sativa* is a wind-pollinated plant that produces large numbers of pollen grains, easily spread over distances of up to 300 km [1-45, 46]. Drifting *Cannabis sativa* pollen could come from multiple sources, including poorly managed medicinal and recreational *Cannabis sativa* farms, Industrial *Cannabis sativa*- hemp farms where male plants are encouraged, or from escaped or naturalized *Cannabis sativa* plants[1-45, 46]. Preventing pollination by drifting pollen can be exceedingly difficult, if not impossible for growers[46]. Therefore, represents a severe limitation to where *Cannabis sativa* can be successfully cultivated [1-45, 46]. Therefore, unintended cross-pollination of *Cannabis sativa* crops is one of the most important threats to cannabinoid production and has been shown to reduce cannabinoid yield [46]. Ploidy manipulation has been used in other crops to improve agronomic traits and reduce fertility[46, 48]. However, little is known about the performance of *Cannabis sativa* polyploids [46, 48]. Polyploidization is considered as a valuable tool in the genetic improvement of crop plants[46-48]. Doubling the chromosome set should allow more flexibility to increase potency or tailor the cannabinoid ratios[48]. The introduction of some of these polyploid traits would be beneficial for the cultivation of cannabis[48]. Cannabis is diploid plant with 20 chromosomes [46-48]. A handful of studies supported the theory that polyploid cannabis might have higher potency, although the results are mixed, with some studies finding decreases in THC [48-53]. However, these studies were conducted with hemp[48].

Polyploidy, the condition of having more than two complete sets of chromosomes, is a widespread and influential phenomenon in the plant kingdom [46, 48-53, 62, 63-89-106-158-166-175]. It has been pivotal in shaping plant evolution, driving biodiversity, and facilitating domestication and improvement in crops[46, 48-53, 62, 63-89-106-158-175]. Whole-genome polyploidization events are central to this process, as polyploid plants often display unique phenotypes, increased vigor, and enhanced adaptability to environmental conditions [46, 48-53, 62, 63-89-106-158-166-174]. These advantages confer a fitness edge to polyploids over their diploid counterparts, influencing a wide array of traits, including morphology, physiology, and stress resilience [46, 48-53, 62, 63-89-106-158-166]. Additionally, the genetic complexity of polyploid genomes supports the production of hybrid proteins and fosters protein diversity, creating a dynamic landscape for evolutionary innovation [46, 48-53, 62, 63-89-106-158-175]. Polyploids generally showed a greater ability to avoid and/or tolerate a range of stress conditions compared with their diploid counterparts, suggesting that polyploidy may confer enhanced flexibility and resilience under climate stress [172]. One of the recent study suggest that polyploidy may provide some resilience to climate change in mixed ploidy populations [172]. However, all species remain susceptible to the impacts of extreme drought and heat stress[172].

Triploid plants are usually both male and female sterile and seeds are not viable or not present [176]. Seedlessness is a highly desirable characteristic for consumers. Triploidy induces the production of seedless fruit and favors vigor [176].Triploid breeding for seedlessness in some ligneous plants such as grapevine or citrus has led to the development of outstanding varieties, some triploid apple or citrus lime have seedy fruits with more limited interest [176] In grape and kiwi, polyploidy increased fruit size [176]. Polyploidization can be used as a breeding tool to modify crop quality[176]. The main effects reported are absence of seeds and modifications in fruit size, shape, and organoleptic quality [176]. Bigger fruit size is very important commercially and is often boosted by cultural practices or chemical treatments[176]. Polyploidization offers a natural and environmentally friendly alternative for increasing fruit size[176]. This is a well-known effect, although the mechanisms behind it continue to be debated today [176]. Final fruit size is determined by coordinated progression of cell production, cell expansion and is correlated with cell size, which is bigger in polyploid [176]. Therefore, application of polyploidization resulting in *Cannabis sativa* triploid and tetraploid plants has been discussed and updated in the following section.

2. *Cannabis sativa:* Diploid Genome

Cannabis sativa is a diploid species with **2n = 20** and genome size is estimated at 818 Mb and 843 Mb for female and male plants, respectively [36, 39-47, 56-61]. According to the karyotype of the species, female plants are homogametic (XX), and males are heterogametic (XY) [36, 39-47, 56-61]. The sex determination of monoecious plants is controlled by homogametic females (XX), but the ratio of male to female flowers is controlled by autosome (XX+A) [36, 39-47, 56]. In this species, the karyotype consists of nine autosomes and a pair of sex chromosomes (X and Y) [56-61]. Female plants are homogametic (XX) and males are heterogametic (XY), with sex determination controlled by an X-to-autosome balance system [57]. The estimated size of the haploid genome of *Cannabis sativa* is 818 Mb for female plants and 843 Mb for male plants, owing to the larger size of the Y chromosome [36, 39-47, 56-61]. Monoecious plants have several agricultural advantages over dioecious ones, such as seed yield, higher crop homogeneity and synchronized maturity [36, 39-47, 56-61]. Monoecious plants can undergo self-pollination and minimize genetic variation [36, 39-47, 56-61]. It has been observed that the sexual phenotype of monoecious plants is unstable [36, 39-47, 56-61]. The multiplication of monoecious seeds in the next generation produces dioecious male and female progenies [36, 39-47, 56-61].

Each cannabis parent plant passes down 10 chromosomes to the offspring, meaning diploid cannabis plant cells possess a total of 2n=20 chromosomes [62]. Researchers believed that diploidy offers organisms somewhat of a survival advantage [62]. Further, two sets of chromosomes mean cells have a software backup if one chromosome gets damaged, but it allows healthy genes to override those with detrimental mutations[62]. In another words, the most cannabis varieties are made up of cells that contain two sets of chromosomes two from each parent [36, 39-47, 56-61, 62]. However, pioneering breeders have created varieties that contain three or even four sets of chromosomes [46, 48-53, 62, 63]. Because these triploid and tetraploid plants produce bigger buds, more phytocannabinoids and terpenes, and larger overall yields [46, 48-53, 62, 63, 89]. Diploidy also offers other benefits [62]. The presence of two sets of chromosomes allows plants to adapt better to their surroundings over time[62]. Inherited sets of genes from both parents also equip plants with genetic variability, increasing the odds of inherited pest and disease resistance and other desirable traits [62]. The vast majority of cannabis cultivars are diploid in nature [36, 39-48, 56-61, 62]. Only a very small percentage of naturally occurring cannabis plants emerge with more than two sets of chromosomes [46, 48-51, 62, 63]. However, cannabis breeders have developed methods to create plants with three or even four sets of chromosomes which is known as polyploidy, and this trait comes with some superb advantages, as well as some disadvantages [46, 48-53, 62, 63].

3. *Cannabis sativa*: Polyploidy

Polyploidization has played a key role in plant breeding and crop improvement. Polyploid plants possess three or more sets of homologous chromosomes [46, 48-53, 62, 63-88, 89-106]. Polyploids are usually infertile with members of their parent species, because diploid X tetraploid crosses produce sterile triploid progeny chromosomes [46, 48-53, 62, 63-89-106]. If the triploid is viable, it is infertile, due to some chromosomes being inherited twice, others once, leading to a lack of gene dosage balance in the gametes chromosomes [46, 48-53, 62, 63-89-106]. One of the reasons why polyploidy is commonest in plants is that they are often hermaphrodite, and hermaphrodites can often self chromosomes [46, 48-53, 62, 63-89-106]. Historically, new cannabis strains have been developed through conventional breeding methods. However, these methods can be imprecise, and required several generations before the desired traits are obtained and a stable strain is produced chromosomes [46, 48-53, 62, 63-89-106]. One strategy to accelerate breeding development is a chromosome doubling event is called as polyploidization [46, 48-53, 62, 63-89-106]. Polyploidy is a significant evolutionary process in plants that involves the duplication of genomic content and has been recognized as a key mechanism driving plant diversification and adaptation. In natural populations, polyploids frequently arise from unreduced gametes, which subsequently fuse with reduced or unreduced gametes, resulting in triploid or tetraploid offspring, respectively[46, 48-53, 62, 63-89-106-175]. In some studies, artificially induced polyploids, and in particular triploids, have been reported to have an increased yield and cannabinoid content [46, 48-53, 62, 63-89-106-1175]. However, other reports indicated that polyploidy has variable impacts on plant growth and development, likely due to the genetic variation in the starting material, and much more work is needed [46, 48-53, 62, 63-89-106-158-175]. Recent research continues to reveal the complexity of polyploid genomes and the diverse ways polyploidy affects plant evolution and adaptation [46, 48-53, 62, 63-89-106-158-165]. Advances in genomics and multi-omics approaches have deepened the understanding of how polyploidization reshapes plant biology at the molecular, physiological, and ecological levels, underscoring its role as a powerful evolutionary force in the plant kingdom [46, 48-53, 62, 63-89-106-158-175].

Polyploidy is an important tool in plant breeding, as it results in increased genetic diversity[46, 48-53, 62, 63-89-106-158-165-175]. The traits of a plant can be altered by changing the chromosomal groups and the number of genes in a

cell, with an outcome that may be desirable or undesirable. Polyploidy occurs in more than 80% of plant species and is responsible for 2–4% of speciation in flowering plants. Domesticated crops such as durum wheat, cotton, tobacco, and potatoes are polyploid organisms, as are ornamental flowers such as violets and lily of the valley[46, 48-53, 62, 63-89-106-158-165-175].

Mutagenic agents such as colchicine, oryzalin, nitroxide, and epoxomicin have been used for polyploidy induction in plants. The most frequently applied is colchicine, although it sometimes has undesirable side effects, including sterility, abnormal growth, chromosomal rearrangement or reduction, and gene mutation [175]. Various explants, such as lateral buds, leaflets, nodules, small branches, tuberous and rhizome fragments, seedlings, sexual or somatic embryos, calli, and cell suspension cultures, have been treated with anti-mitotic agents to generate polyploid plants[175]. During *in vitro* induction, the culture media can be solid, semi-solid, or liquid, depending on the plant species and anti-mitotic agent[175]. The duration of the treatment also depends on the anti-mitotic agent used, the plant species, the culture medium, and the explant [175].

Polyploidy is thought to be an evolutionary and systematic mechanism for gene flow and phenotypic advancement in flowering plants[46, 48-53, 62, 63-89-106-158-175]. It is a natural phenomenon that promotes diversity by creating new permutations enhancing the prime potentials as compared to progenitors [168]. Two different pathways have been recognized in studying polyploidy in nature; mitotic or somatic chromosome doubling and cytogenetics variation[46, 48-53, 62, 63-89-106-158-168]. Secondly, the vital influence of being polyploid is its heritable property (unreduced reproductive cells) formed during first and second-division restitution (FDR & SDR) [46, 48-53, 62, 63-89-106-158-168]. Different approaches either chemical (Colchicine, Oryzalin, Caffeine, Trifuralin, or phosphoric amides) or gaseous i.e. Nitrous oxide have been deliberated as strong polyploidy causing agents[168]. A wide range of cytogenetic practices like chromosomes study, ploidy, genome analysis, plant morphology and anatomy have been studied in different plant species[168]. Flow cytometry for ploidy and chromosome analysis through fluorescence and genomic *in situ* hybridization (FISH & GISH) are the basic methods to evaluate heredity substances sampled from leaves and roots[168]. Many horticultural crops have been developed successfully and released commercially for consumption[168]. Moreover, some deep detailed studies are needed to check the strong relationship between unique morphological features and genetic makeup concerning genes and hormonal expression in a strong approach[168].

Polyploidization is a common in the plant kingdom and has been associated with increased genetic diversity in some plant lineages [46, 48-53, 62, 63-88-106]. Desirable consequences of polyploidy for plant breeding include the buffering of deleterious mutations, increased heterozygosity, and hybrid vigor [46, 48-53, 62, 63-88-106]. Consequently, polyploids often have phenotypic traits that are distinct from diploids, including larger flowers or leaves [46, 48-53, 62, 63-88-106-158]. Increase in active metabolite concentration in tetraploids are reported for numerous medicinal plants including *Artemisia annua, Papaver somniferum, Datura stramonium, Thymus persicus, Echinacea purpurea,* and *Tanacetum parthenium* [46, 48-53, 62, 63-88-106]. Somatic doubling or the generation of unreduced gametes are the main mechanisms known to result in natural polyploidy [46, 48-53, 62, 63-88-106-175]. Somatic doubling is linked to mitotic processes (e.g., endoreduplication or endomitosis), which can take place within apical meristematic tissues or zygote cells, ultimately leading to the development of entirely polyploid organisms or mixoploid individuals [46, 48-53, 62, 63-88-106-160]. However, it is widely acknowledged that somatic doubling is of relatively minor significance in the natural formation of polyploids, despite this being the primary mechanism used for the induction of artificial polyploids[46, 48-53, 62, 63-88-106-175].

Polyploidization occurs through two principal mechanisms: autopolyploidy, which results from genome duplication within a single species, and allopolyploidy, arising from hybridization between different species followed by genome doubling[46, 48-53, 62, 63-88-106-175]. These genome duplication events act as significant genomic shocks, initiating widespread genome rearrangements, alterations in gene regulation, and shifts in the epigenetic landscape [46, 48-53, 62, 63-88-106-175]. Furthermore, polyploidization stimulates transposable element activity, driving additional genomic changes that contribute to the evolutionary trajectory of polyploids [46, 48-53, 62, 63-88-106-170].

Polyploidy is a cellular state containing more than two complete chromosome sets. It has largely been studied as a discrete phenomenon in either organismal, tissue, or disease contexts[46, 48-53, 62, 63-88-106-170]. Ploidy manipulation is a valuable tool in plant breeding [46, 48-53, 62, 63-88-106-170]. Important consequences of genome doubling can include larger organs and improved production of secondary metabolites, often linked to increased tolerance to biotic and abiotic stress [46, 48-53, 62, 63-88-106]. Polyploid forms also provide a wider germplasm base for breeding [46, 48-53, 62, 63-88-106-160]. Polyploids have yet to be implemented in most breeding programs for cannabis [1-46, 48-51, 61, 62, 63]. The treatment of axillary buds with the dinitroaniline herbicide oryzalin is an effective method for chromosome doubling [1-46, 48-51, 61, 62, 63]. Past studies on the polyploidization of hemp and its closest relative hops (*Humulus lupulus* L.) used colchicine for doubling [1-46, 48-51, 61, 62, 63].

Numerous studies have highlighted the influence of geographical, environmental, agroclimatic, and genetic factors in shaping both the quantity and quality of secondary metabolite production in plants [173]. Synthetic polyploids demonstrated superior adaptability across diverse environments compared to diploids, which can influence the screening of superior polyploid genotypes [173]. So, analyzing the interaction between genotypes and various environmental factors is crucial [173]. Additionally, synthetic polyploidization can affect the selection of desired genotypes through epigenetic instability, disrupted genomic imprinting, and unwanted epistasis[173]. Epigenetic instability can cause variable gene expression, complicating trait selection. Disrupted genomic imprinting may lead to inconsistent trait outcomes, while unwanted epistasis can obscure genotype-phenotype relationships, making it challenging to stabilize and select beneficial traits[173]. These complexities need to be managed to effectively achieve the desired polyploid genotypes[173].

The predominant mechanism leading to polyploidy in plants is understood to involve the production and subsequent fusion of unreduced gametes [46, 48-53, 62, 63-88-106-160]. In this instance, the unreduced gamete is diploid, containing two copies of each gene, instead of the standard haploid state with only one copy[46, 48-53, 62, 63-88-106-160]. The ability to generate unreduced reproductive cells is a hereditary trait observed in numerous plant species [46, 48-53, 62, 63-88-106-175]. In addition to genetic regulation, environmental factors (e.g., nutrient deficiencies, water scarcity, injury, herbivory, and temperature) can exert an influence on unreduced gamete formation [46, 48-53, 62, 63-88-106-160]. Once created, the unreduced gamete possesses the capacity to merge with another unreduced gamete to produce a tetraploid, a process known as bilateral polyploidization, or with a gamete that has undergone reduction to produce a triploid, termed unilateral polyploidization[46, 48-53, 62, 63-88-106-175].

The increase in chromosome number in plants is the result of a genome duplication event chromosomes [46, 48-53, 62, 63-88-106]. Depending on the origin of the genome duplication event, there is considered to be two different polyploid types: autopolyploids, which are derived from multiplication of a diploid genome (intraspecies), and allopolyploids, which are the consequence of hybridization followed by doubling of the two haploid genomes (interspecies; Comai, 2005) [84]. Despite this difference, both types of polyploids profit from a genomic buffering effect provided by the doubling of their genetic information [84]. A polyploidy occurs in almost all flowering plants to varying degrees [46, 48-53, 62, 63-88-106]. Many of the agricultural crops that have become staples in the human diet are polyploids, including strawberries, coffee, potato, and oats [46, 48-53, 62, 63-88-106]. In addition, the combination of different genomes in allopolyploids results in changes in genome organization and altered gene expression of parental genomes, putatively causing additive heterosis effects [84]. Although still not fully understood, the increase in chromosome number and additional genomic interactions and genetic alterations often results in superior properties in polyploid plants compared to those in their diploid counterparts, making polyploidization a credible approach for crop improvement [46, 48-53, 62, 63-88-106]. Polyploidization has already been successfully implemented in plant breeding programs to increase overall yield and biomass of several crop species, including potato, red clover, sugar beet, watermelon, etc [46, 48-53, 62, 63-88-106]. Several studies suggest that an increased somatic ploidy level may influence biomass composition in a way that could be beneficial for the production of bio-energy [84, 107]. In addition, higher ploidy plants displayed altered sugar composition [84]. Such effects were linked to the delayed development of polyploids [84]. Moreover, the changes in polyploid cell wall composition promoted saccharification yield [84]. The results of study by Corneillie et al,, (2019) [84] indicated that induction of polyploidy is a promising breeding strategy to further tailor crops for biomass production [84].

Cannabis breeders have figured out ways to increase the number of chromosome sets within cannabis cells, using both chemical intervention and crossbreeding between diploid and polyploid varieties [46, 48-51, 61, 62, 63]. Polyploid cannabis varieties differ from their diploid counterparts in several ways, including: **Cell wall composition:** Research showed that polyploidy changes the composition of the cell wall the structure that surrounds the plasma membrane [46, 48-51, 62, 63, 84, 107]. These changes influence the levels of proteins and complex carbohydrates in the cell wall, which can have a big influence on drought tolerance and pathogen defense[46, 48-51, 61, 62, 63]. **Biomass production:** Polyploid hemp produces more biomass and heavier flowers[46, 48-51, 61, 62, 63]. This has important implications in commercial agriculture for both industrial hemp and medicinal and recreational cannabis[46, 48-51, 61, 62, 63]. **Less fertile:** Polyploids are often infertile [46, 48-51, 61, 62, 63]. This might sound like a disadvantage, but it actually comes in handy when growing seedless weed flowers [46, 48-51, 61, 62, 63]. Naturally occurring triploids, but no tetraploids, were identified at a low rate in most cannabis populations[158-160]. These results of the work by Philbrook et al., (2023) [158] clearly demonstrated that polyploidy is a naturally occurring phenomenon in cannabis, likely as a result of unreduced gametes[158]. These results have significant implications for future breeding efforts and help to explain the presence of polyploids within landrace populations and commercial production [158].

As legalization continues to blossom, research and development efforts are ramping up and cannabis companies are pouring considerable time, effort, and money into creating cannabis plants/products that increase profits and better

satisfy customer demands [1-46, 48-51, 61, 62, 63]. So far, breeders have created cultivars with higher levels of THC, bigger buds, higher yields, and more flavor [46, 48-51, 61, 62, 63]. Now, researchers are applying the concept of polyploidy to improve cannabis as an agricultural crop [46, 48-51, 61, 62, 63]. Through chemical treatments and crossbreeding, the following types of cannabis polyploids have arisen [46, 48-51, 61, 62, 63]. T**riploid cannabis:** As the name suggests, triploid cannabis contains three sets of chromosomes which adds up to 30 in total [46]. The offspring inherit a single set from one parent and two from the other[46]. **Tetraploid cannabis:** These cultivars possess four sets of chromosomes, two from each parent[48]. Although tetraploid plants possess more copies of DNA, they are a precursor to triploid plants during the breeding process[48].

Polyploid cannabis plants offer a host of benefits, and they may help growers to overcome the fertilization issue when it comes to cultivating cannabis on a large scale[46, 48-51, 61, 62, 63]. As a dioecious plant, cannabis emerges from seed as either a distinct male or female specimen [1-46, 48-51, 61, 62, 63]. The females produce resinous flowers high in cannabinoids and terpenes (if left unfertilised), whereas the male flowers produce pollen[1-46, 48-51, 61, 62, 63]. Overall, male plants are relatively worthless when it comes to cannabis crop production[1-46, 48-51, 61, 62, 63]. Not only that, but they decrease the quality of female flowers upon fertilization, turning them from sweet sinsemilla into seedy and less potent buds[1-46, 48-51, 61, 62, 63]. The current cannabis industry standard for dealing with this issue involves genetic testing before transplanting[1-46, 48-51, 61, 62, 63]. However, this quickly becomes costly and time-consuming. However, both feminized seeds and cloning offer an alternative solution[1-46, 48-51, 61, 62, 63]. Polyploid cannabis could become another option for commercial and small-scale growers looking to produce seedless weed [1-46, 48-51, 61, 62, 63]. The introduction of some of these polyploid traits would be beneficial for the cultivation of cannabis[1-46, 48-51, 61, 62, 63].

Cannabis is diploid plant with 20 chromosomes [1-46, 48-51, 61, 62, 63]. Doubling the chromosome set should allow more flexibility to increase potency or tailor the cannabinoid ratios[1-46, 48-51, 61, 62, 63]. A handful of studies supported the theory that polyploid cannabis might have higher potency, although the results are mixed, with some studies finding decreases in THC [1-46, 48-51, 61, 62, 63]. However, these studies were conducted with hemp. The effects of polyploidization on drug-type cannabis strains is unknown [1-46, 48-51, 61, 62, 63]. Polyploidy can be induced through application of antimitotic agents to seeds, seedlings, in vivo shoot tips, or *in vitro* explants [46, 48-53, 62, 63-88-106]. However, drug-type cannabis strains are not genetically stable when propagated through seeds, and while there has been little success in regenerating cannabis shoots from callus, the propagation of high THC drug-type cannabis in tissue culture using nodal explants has been described [1-46, 48-54, 61, 62, 63]. These plants have been shown to be genetically and chemically stable through 30 rounds of tissue culture propagation [1-46, 48-54, 61, 62, 63]. One of the study by Parsons et al., (2019) [48] described an effective method for generating cannabis tetraploids from axillary bud explants and the subsequent analysis of polyploidy effects on growth, yield, and phytochemistry in a drug-type cannabis strain[1-46, 48-54, 61, 62, 63]. This research lays important groundwork for the development of improved cannabis strains and novel germplasm for breeding efforts [1-46, 48-51, 61, 62, 63].

Polyploidy plays an important role in plant diversification and speciation [175]. The ploidy level of plants is associated with morphological and biochemical characteristics, and its modification has been used as a strategy to alter the quantitative and qualitative patterns of secondary metabolite production in different medicinal plants[175]. Polyploidization can be induced by many anti-mitotic agents, among which colchicine, oryzalin, and trifluralin are the most common[175]. Other variables involved in the induction process include the culture media, explant types, and exposure times. Due to the effects of polyploidization on plant growth and development, chromosome doubling has been applied in plant breeding to increase the levels of target compounds and improve morphological characteristics[175].

One of the study by Parsons et al., (2019) [48] described the development of tetraploid drug type cannabis lines and test whether this transformation alters yield or the profile of important secondary metabolites: Λ9 - tetrahydrocannabinol (THC), cannabidiol (CBD), or terpenes[48]. The mitotic spindle inhibitor oryzalin was used to induce polyploids in a THC/CBD balanced drug-type strain of *Cannabis sativa*[48]. Cultured axillary bud explants were exposed to a range of oryzalin concentrations for 24 h [48]. Flow cytometry was used to assess the ploidy of regenerated shoots [48]. Treatment with 20–40 mM oryzalin produced the highest number of tetraploids[48]. Tetraploid clones were assessed for changes in morphology and chemical profile compared to diploid control plants[48]. Tetraploid fan leaves were larger, with stomata about 30% larger and about half as dense compared to diploids[48]. Trichome density was increased by about 40% on tetraploid sugar leaves, coupled with significant changes in the terpene profile and a 9% increase in CBD that was significant in buds[48]. No significant increase in yield of dried bud or THC content was observed[48]. This research lays important groundwork for the breeding and development of new cannabis strains with diverse chemical profiles, of benefit to medical and recreational users[48].

Oryzalin is a potent herbicide that inhibits microtubule polymerization to promote polyploidization [1-46, 48-51, 61, 62, 63]. However, oryzalin has greater specificity for plant tubulins and is considered a more effective and less toxic alternative to colchicine [46, 48-53, 62, 63-88-106]. Trojak-Goluch and Skomra (2013) found that 125 mM of colchicine applied to explants was the most effective for polyploidization of hops[46, 48-53, 62, 63-88-106]. The concentrations in the range of 20 and 40 mM were the most effective for tetraploidization of cannabis, indicating that oryzalin is effective at over 30 times lower concentration compared to colchicine [1-46, 48-51, 61, 62, 63]. According to the study reported by Parsons et al., (2019) [48], two *Cannabis sativa* strains were tested: one THC dominant indica strain (strain 1), and one balanced THC/CBD indica dominant hybrid strain (strain 2) [48]. Axillary buds treated with high concentrations of oryzalin had a poor survival rate[48]. No explants survived the 150 mM treatment [48]. Survival rates for explants treated with 20 mm oryzalin ranged from 62.5% to 87.5% for strain 1 and 2, respectively[48]. Strain 1 *Cannabis indica* was less tolerant of oryzalin treatment compared to strain 2 *Cannabis sativa* and yielded a higher ratio of mixoploids [1-46, 48-51, 61, 62, 63]. Similar genotype differences in response to oryzalin treatment have been found in other species such as cherry laurel and Japanese quince [46, 48-53, 62, 63-88-106]. The two tetraploids of strain 1 that were isolated did not easily regenerate shoots on the current media. Compared to strain 2 *Cannabis sativa* tetraploids, these plants were sickly and slow-growing[46, 48-53, 62, 63-88-106]. This response could reflect a greater sensitivity to oryzalin treatment or polyploidization may alter media requirements or hormone concentrations necessary to grow shoots[46, 48-53, 62, 63-88-106]. Overall, clone health and survival was lower among tetraploid clones, possibly due to lower rooting success[46, 48-53, 62, 63-88-106-175]. This finding matches with hops, whose tetraploids also have slower root development in culture and difficulty acclimating to a greenhouse environment [46, 48-53, 62, 63-88-106]. Despite these early difficulties, tetraploid strain 2 *Cannabis sativa* plants grew and flowered at a rate comparable to diploids, yielding a similar amount of dried bud [48]. On the other hand, data suggests that tetraploidization of cannabis hinders rooting but has no significant negative effect on overall plant growth or yield[1-46, 48-51, 61, 62, 63].

4. *Cannabis sativa*: Triploid varieties

Triploid cannabis varieties are practically infertile and rarely produce seeds, regardless of whether they are surrounded by pollen-producing males [1-46, 48-51, 61, 62, 63]. Their odd sets of chromosomes impair the cellular processes that lead to seed development [46, 48-51, 61, 62, 63]. Triploid plants are not exclusive to the world of cannabis breeding[46, 48-51, 61, 62, 63]. Horticulturalists have developed triploid varieties across a host of different species to reap their benefits [46, 48-53, 62, 63-88-106]. For example, seedless fruit, such as watermelons and bananas, are triploid [46, 48-53, 62, 63-88-106-157]. Because they can not produce seeds, they are more convenient to consume and therefore, more appealing to consumers[46, 48-53, 62, 63-88-106]. Much like other triploid crops, cannabis strains that possess three sets of chromosomes also offer key benefits[46, 48-51, 61, 62, 63]. They create more biomass in terms of leaves and stems, which makes them more valuable in some commercial settings [46, 48-51, 61, 62, 63]. Larger flowers also make them extremely appealing to growers, as well as customers seeking big buds in dispensaries and other retail outlets [46, 48-51, 61, 62, 63]. Triploid plants do occur naturally, but they are extremely rare [46, 48-53, 62, 63-88-106-174]. In order to create them, breeders need to first create tetraploid weed cultivars[46, 48-53, 62, 63-88-106-157]. Once they secure tetraploid genetics, breeders then need to select suitable diploid specimens with desirable traits[46, 48-53, 62, 63-88-106]. Crossing a carefully selected tetraploid parent with a diploid parent will create triploid offspring [46, 48-53, 62, 63-88-106-174].

The breeding of triploid cultivars has been used as a strategy to produce non-invasive or infertile cultivars in many crops, including Acer spp. [64], *Humulus lupulus* [65], *Hypericum androsaemum* [66], and *Miscanthus sinensis* [67]. Reports have speculated that triploid *Cannabis sativa* cultivars may be infertile, and therefore, resistant to the yield damage caused by pollination [46, 48-51, 61, 62, 63]. However, were unable to locate studies demonstrating triploid hemp sterility [46, 48-51, 61, 62, 63]. Several other advantages to ploidy manipulation of crop species have been documented in the literature [46, 48-53, 62, 63-88-106]. Specifically, ploidy manipulation has been shown to increase secondary metabolite yields in several medicinally important or essential oil crops, such as *Papaver bracteatum* [68], *Lavandula vera* [69], *Echinacea purpurea* [70], and *Cannabis sativas* closest relative of economic importance, *Humulus lupulus* [65]. Furthermore, polyploid versions of several ornamental plant species, such as the interspecific hybrids of poinsettia (*Euphorbia pulcherrima* X *E. cornastra*) [71], and monk verbena (*Glandularia peruviana* X *G. scrobiculata*) [72], and hybrids of myrtle (*Lagerstroemia indica*) [73], impatiens (*Impatiens balsamina*) [74, 75], and hibiscus (*Hibiscus rosa-sinensis*) [76], have been shown to display significantly larger flowers than their diploid counterparts [46, 48-53, 62, 63-88-106-174].

Triploid plants have larger organs, greater biomass, and strong stress resistance by preserving relatively larger amounts of photosynthetic energy[46, 48-53, 62, 63-88-106-174]. The undesirable spread of non-native invasive crop and horticultural plants into natural areas can also be reduced or eliminated by the use of triploids, because they tend to be sterile and seedless[46, 48-53, 62, 63-88-106-174]. Triploid plants have great economic value and have been useful for

developing new agronomic, horticultural, and forestry plant varieties[46, 48-53, 62, 63-88-106-174]. Because of rapid advances in DNA sequencing technology, triploids may become a focus of genomic research in the future, and will create unprecedented opportunities for discovering and monitoring genomic and transcriptomic changes in unbalanced genomes [46, 48-53, 62, 63-88-106-174]. One of the new trends in genomics research is to create synthetic triploid plants as materials for the study of first genomic responses that occur immediately after triploid formation[46, 48-53, 62, 63-88-106-174].

Triploid plants have three sets of chromosomes, and many desirable characteristics, including greater vigor; broad, thick, dark green leaves; and larger flowers or fruit, which results in higher yield or higher harvest index[46, 48-53, 62, 63-88-106]. For example, the Vertigo watermelon variety (2n = 3x = 33) has produced the highest watermelon yields (41 000 lb/acre) [46, 48-53, 62, 63-88-106-157]. Triploid cassava also has a high yield with outstanding culinary and industrial qualities [46, 48-53, 62, 63-88-106-157]. Triploid plants produce seedless fruits in different species like citrus, banana and watermelon[46, 48-53, 62, 63-88-106-157]. Only in citrus, international markets demand fruits without seeds and this characteristic is one of the most important for citrus with special emphasis in mandarins[46, 48-53, 62, 63-88-106-157]. Sterile triploid crop and horticultural plants can reduce or eliminate the undesirable spread of non native invasive crop plants that produce numerous seeds into natural areas[46, 48-53, 62, 63-88-106-157]. Thus, triploid plants will play an even more important role in agriculture, forestry, and ecology in the future[46, 48-53, 62, 63-88-106-157-161].

One of the new trends in genomic research is to create synthetic polyploid plants to provide materials for studying initial genomic responses immediately after polyploid formation[46, 48-53, 62, 63-88-106-157]. Thus triploid plants have attracted more attention and there has been recently great progress in understanding the details of their formation after decades of investigation [46, 48-53, 62, 63-88-106-157]. The applications of triploid plants, ways to generate triploid plants, possible obstacles to generating triploids, and some solutions to these obstacles has been updated [46, 48-53, 62, 63-88-106-157-161].

Errors occur sometimes during meiosis in regular diploid plants and chromosomes fail to segregate properly to the daughter cells[85]. Such an unreduced 2n gamete can unite with a normal, haploid gamete, resulting in a triploid zygote that may develop into a triploid plant [85]. Triploid cells have three complete sets of chromosomes, and are designated 3n[85]. When meiosis occurs, the probability of obtaining 2n and n gamete is only (1/2) x–1[85]. For chromosome numbers x > 8, this probability is reduced by 1% [85]. Hybridization between one parent with unreduced gametes (2n gametes) and another diploid parent is the typical way to triploid formation [85]. Both 2n megagametophyte and 2n microgametophyte occur in both wild and cultivated hybrid and non-hybrid species[85]. There are four mechanisms by which triploids form in addition to somatic fusion. The female parent with unreduced gametes plays particularly important role in triploid plant formation [46, 48-53, 62, 63-88-106-157]. Two sets of chromosomes in the triploid plant are derived from the female parent [85]. Further, the triploid embryo needs nutrition provided by endosperm, which consists of two polar nuclei from female parent and sperm cell from the male parent[85]. Successful hybridizations between different mating types showed that 2n female gamete is more efficient than the 2n pollen for the formation of triploid plants during hybridization [85]. Embryo-endosperm balance number can determine the viability of seeds and the exit of cross direction. These various triploid formation mechanisms results in different levels of offspring fertility and phenotypes[46, 48-53, 62, 63-88-106-157-161-174].

Several ploidy manipulations in *Cannabis sativa* have been reported in the literature [46, 48-51, 61, 62, 63]. However, knowledge of the effects on chemical profiles is limited to the differences between tetraploid and diploid type I and type II plants, that are tetrahydrocannabinol (THC)- dominant, or those that produce both THC and cannabidiol (CBD), respectively [46, 48-51, 61, 62, 63]. These studies indicated that although THC concentration remained unchanged in polyploids, CBD production was slightly increased [46, 48-51, 61, 62, 63]. A method of producing triploid type III plants, those that are CBD-dominant, has also been reported [46, 48-51, 61, 62, 63]. However, the field phenotype and fertility status of the triploid plants were not discussed[46, 48-51, 61, 62, 63]. Furthermore, they were unable to locate any studies discussing the impact of ploidy manipulation on type IV, cannabigerol (CBG)-dominant, cultivars[46, 48-51, 61, 62, 63]. Given the potential advantages of ploidy manipulation in *Cannabis sativa*, the primary goal was to produce and test the fertility and cannabinoid content of diploid, triploid, and tetraploid type IV *Cannabis sativa* plants [46, 48-51, 61, 62, 63].

In one of the study reported by Crawford et al., (2021)[46], a colchicine treatment was used to produce tetraploid versions of two CBG-dominant inbred lines, 'TS1-3' and 'P163'. Diploid (2x), triploid (3x), and tetraploid (4x) F1 hybrid seeds were produced from crossing the combinations TS1-3 (2x) X P163 (2x), TS1-3 (4x) X P163 (2x), and TS1-3 (4x) X P163 (4x) [46]. Several crosses between these F1 hybrids were attempted to determine intraploid compatibilities[46]. The seed number produced by each pollinated plant was counted[46]. The cannabinoid yield, dry biomass, inflorescence

weight, and cannabinoid concentrations of different ploidy hybrid plants, grown both indoors and outdoors, were also measured [46]. Several methods of ploidy manipulation in *Cannabis sativa* were published prior to this study [46, 48-51, 61, 62, 63]. However, this is the first report that tracks phenotypic differences between diploids, triploids, and tetraploids in relation to seed production, biomass, and cannabinoid yield [46]. This study also further reported the crossing compatibility between these ploidies [46]. The plants used in this study were from a single genotype, and in the case of the clonally propagated TS1-3, only one colchicine treated individual was used in the trials [46]. Off-target mutations not identified by flow cytometry, including smaller (non-chromosome level) deletions or insertions, or other effects independent of genome doubling, are known to occur as a result of colchicine treatments [46]. For example, in poinsettia, various morphological mutations were observed following treatment with colchicine [71]. In Arabidopsis, colchicine treatment also resulted in plant performance differences in their progenies [82]. Therefore, repeating these trials with several different colchicine-treated individuals of the same genotype would help to elucidate the reproducibility of the results[46]. Ploidy manipulations, using both colchicine and oryzalin, have been reported in *Cannabis sativa* [46, 48-51, 61, 62, 63]. However, the non-ploidy mutation effects of these chemicals remain unknown [46]. Further research on specific chemicals and concentrations, and their effects on off-target mutations in the hemp genome, would be beneficial for the breeding of polyploid cannabis[46, 48-51, 61, 62, 63]. Additional research investigating the effect of polyploidy in cannabis breeding, which includes several additional genotypes and reciprocal crosses, would also be valuable in understanding the applicability of the results described in this study to the species as a whole[46, 48-51, 61, 62, 63]. According to the study reported by Crawford et al., (2021) [46] only included one genotype of an F1 hybrid triploid from a single directional cross, due to limitations in pollen viability[46]. Research that includes multiple genotypes is needed to understand the interactions between ploidy and genotype [46]. Comparisons using diploids and triploids, each produced from reciprocal crosses, will help to elucidate quantitative trait improvements between specific parental gene doses and the effects of polyploidy[46]. The effect of chromosome doubling in the parents would also be valuable in future studies to help detangle the effects of hybridization versus polyploidy [46]. A final future area of investigation may be into the use of true male pollen in testing the sterility of the triploid *Cannabis sativa* plants [46]. Previous research has indicated that the pollen from reversed female *Cannabis sativa* plants is frequently less viable than the pollen from true genetic males [83]. Since pollen from true males is a contaminant problem from fiber and grain crops, these additional experiments are warranted to ensure that triploid plants will maintain their sterility in field conditions[46]. Understanding the dosage and timing of pollination may also be valuable to confirming the value of using triploids in essential oil crops [46].

This study reported by Crawford et al., (2021) [46] showed the potential for ploidy manipulation in the improvement of hemp cultivar biomass and cannabinoid yield [46]. The triploid CBG-dominant F1 hybrid plants showed higher biomass, inflorescence weights, and cannabinoid concentrations as compared to the diploid plants[46]. Although the differences in cannabinoid concentrations and total CBG(A) concentration were not statistically significant between the diploids and triploids, the approximately 1.5% increase in each may be of economic importance to growers[46]. Notably, the total THC(A) concentration did not increase with the total CBG(A) concentration and ploidy[46]. These results mimic those shown in other studies on the ploidy manipulation of *Cannabis sativa* [46, 48-51, 61, 62, 63]. These results are also valuable to breeders and growers interested in triploid *Cannabis sativa* cultivars, given the current strict limitations on THC(A) content in industrial hemp plants and plant products[46, 48-51, 61, 62, 63].

Cannabis sativa L. is a diploid species, but recent work using artificially induced polyploidy has demonstrated its potential advantages in an agricultural setting[158]. Further, recent work has identified that some elite clonal cultivars, vis. Mac1, are triploid, with no indication that they were artificially produced[158]. One of the study conducted by Philbrook et al., (2023) [158] reported that if polyploidy is a naturally occurring phenomenon in cannabis and estimated the frequency of this phenomenon across populations[158]. To do this, the presence of natural triploid individuals was evaluated in 13 seedling populations of cannabis using a flow cytometry analysis[158]. Among the examined populations, natural triploids were identified in 10 groups with an average frequency of approximately 0.5%[158]. According to the study conducted by Philbrook et al., (2023) [158], the highest frequency of natural triploids was observed in a self-pollinated population at 2.3% [158]. This research demonstrated that polyploidy is a naturally occurring event in cannabis and triploids are present at an average of approximately 0.5%, or 1 in 200 plants [158]. These data shed light on the natural variation in ploidy within cannabis populations and contributed valuable insights to the understanding of cannabis genetics and breeding practices [158]. These findings underscore the prevalence of natural triploidy in cannabis populations and emphasize its relevance in the field of cannabis genetics [158]. It is apparent that future investigations should focus deeper into the mechanisms underpinning triploidy in cannabis and its potential implications for cannabis breeding and cultivation practices[158]. Of particular importance is the need to explore triploid cannabis sterility and seed development, shedding light on the reproductive biology of the species and helping to develop truly seedless individuals [158]. Therefore, the discovery of natural triploidy in cannabis opens up exciting avenues for further research, offering insights that may revolutionize the cannabis industry and its scientific understanding[158].

While cannabis is typically a diploid species, instances of presumably natural triploid (3x) [159] and tetraploid (4x) [160] plants have been observed in some studies[158]. Further, some elite clonal cultivars used in commercial production have been identified as triploids (i.e., Mac1), with no indication that they were artificially produced (unpublished data) [158]. Despite their rarity, naturally occurring polyploid cannabis plants offer valuable insights into the plant's genetic diversity and adaptation to varying environmental conditions [158, 159]. Understanding the genetic consequences of natural polyploidy in cannabis is of significant interest to both researchers and breeders [46, 48, 158]. These polyploid individuals may exhibit distinct traits, such as altered morphology or growth patterns, which could have implications for their ecological niche or potential utility in cultivation [46, 48, 156]. Furthermore, investigating the stability and reproductive viability of naturally occurring polyploid cannabis plants is essential for comprehending the long-term evolutionary dynamics of this species [158, 159]. Research in this area can provide valuable insights into the adaptive potential of cannabis populations and inform breeding efforts aimed at improving various aspects of cannabis cultivation, including the yield, cannabinoid content, and environmental resilience [46, 48-51, 61, 62, 63, 158].

5. *Cannabis sativa*: Traits of Triploid

The triploid and diploid plants differ genetically, there are differences expressed, The key differences exhibited by triploid plants include: **Larger and more Growth:** Research showed that triploid cannabis plants tend to grow larger than their diploid counterparts[46, 48-51, 61, 62, 63]. An overall increase in biomass makes for bigger fan leaves, longer stems, and a more extensive root network in the soil [46, 48-51, 61, 62, 63]. **Slower to grow:** The size and productivity of triploid plants come with a trade-off, they take longer to grow. While not a problem for hobbyists, the length of the growing cycle poses an issue for commercial ventures that value a quick turnaround[46, 48-51, 61, 62, 63]. **More cannabinoids:** Studies analyzing the chemical qualities of diploid and polyploid weed strains have found higher levels of cannabinoids in triploid varieties[46, 48-51, 61, 62, 63]. **Bigger buds:** Triploid cannabis varieties also produce bigger flowers. The buds are longer, wider, and denser[46, 48-51, 61, 62, 63]. Larger buds and more cannabinoids make for dramatically larger yields per plant. Triploid cannabis opens up a whole new field of play for breeders [46, 48-51, 61, 62, 63]. Just like the advent of autoflowering, feminized genetics, a rise in triploid plants could change the cannabis industry in many ways[46, 48-51, 61, 62, 63]. Contemporary research has found that these varieties certainly showed promise [46, 48-51, 61, 62, 63]. However, this innovation is still in the early stages, and triploid weed plants also have some downsides that could affect their adoption among breeders and growers, both commercial and amateur[46, 48-51, 61, 62, 63]. A breeder could cross diploid and triploid parent strains. However, the results probably would not be worth the effort, as their unequal amount of chromosome sets can disrupt the normal reproductive process[46, 48-51, 61, 62, 63]. On top of that, triploids are almost always sterile, meaning there is only a very tiny chance that they will go to seed to produce offspring[46, 48-51, 61, 62, 63].

Although one of the chief characteristics of true triploids is partial or total sterility, this sterility can be horticulturally useful[46, 48-51, 61, 62, 63]. Flowers of triploid plants are generally larger and more colorful than those of their diploid counterparts partly because the energy that is normally devoted to seed formation is used for flowers or other organs[46, 48-51, 61, 62, 63]. Triploid flowers often have longer shelf life and the triploid plants require little or no 'dead-heading' (the removal of faded or dead flowers from plants to maintain both a plant's appearance and to improve its overall flowering performance) [46, 48-51, 61, 62, 63].

Neutralizing invasive plants. Weedy invasive plants have been a problem in the United States for years. The first comprehensive assessment of weedy invasive plants in the continental United States has found that non-native plants are more widely distributed than are native plants (http://news.sciencemag. org/biology/2015/01/invasive-plants-taking-over-us) [46, 48-51, 61, 62, 63-106]. Gene flow mediated by pollen has also been demonstrated between commercial cultivars and weedy relatives [46, 48-51, 61, 62, 63]. Thus, sterile triploid cultivars can be a vital strategy for reducing the invasiveness of crop plants[46, 48-51, 61, 62, 63]. Many invasive plant species are considered noxious because they produce massive amounts of seeds, which can be dispersed by birds or other means and colonize surrounding areas of native flora, resulting in major transformation of ecosystems such as forests, roadsides, parks, preserves, wildlife refuges, and urban areas[46, 48-51, 61, 62, 63]. However, if this seed production can be blocked, these plants may behave well as crops or high-quality ornamentals without this invasive tendency[46, 48-51, 61, 62, 63]. One potential solution good for both the horticultural industry and for the environment is to create seedless versions of plants that have been shown to be, or that have potential to be invasive[46, 48-51, 61, 62, 63]. Thus, seedless triploid varieties can play an important role in neutralizing the invasiveness of introduced plants[46, 48-51, 61, 62, 63].

Triploid production increases the size of somatic cells and guard cells (and increases chloroplast number, which results in strengthening photosynthesis [46, 48-51, 61, 62, 63-106-157-174]. Therefore, many triploid plants are relatively more vigorous; have short internodes; broad, thick, dark green leaves, resulting in greater biomass or crop yield per

plant[46, 48-51, 61, 62, 63-106-174]. Hoshino et al. (2011) found that triploids, including cassava (*Manihot esculenta* C.), watermelon, little gourd (*Coccinia grandis* (L.) J. Voigt), had higher yields and higher starch content[46, 48-51, 61, 62, 63-106-157]. Today, over 80% of the watermelons produced in the US are seedless triploid (www.watermelon.org) [46, 48-51, 61, 62, 63-106-157]. The triploid seedless watermelon commands premium prices because of its high quality flesh that is virtually free of seeds [46, 48-51, 61, 62, 63-106]. The protein content of triploid mulberry leaves is 4.14% higher than that of diploid mulberry [46, 48-51, 61, 62, 63-106-157]. Therefore, the edibility and digestibility of triploid mulberry leaves is higher for silkworms[46, 48-51, 61, 62, 63-106-157]. When fed triploid mulberry leaves, silkworms grow more rapidly, which reduces the length of their life cycle by about 2–3 days and increases whole cocoon weight, cocoon layer weight, and pupal weight over those fed with diploid leaves [46, 48-51, 61, 62, 63-106-157]. Cocoon production is also increased by 14–16%, and fecundity improved by about 11% [46, 48-51, 61, 62, 63-174]. As in these examples, the use of triploid plants can result in economic benefits in several kinds of farming systems [46, 48-51, 61, 62, 63-106-174].

Triploid plants can also be produced by natural selection, sexual hybridization, endosperm in vitro culture and fusion of somatic diploid protoplasts with haploid microspore cells [46, 48-55, 62, 63-88-106-157]. There are few reports on protoplast fusion to produce triploid plants [46, 48-53, 61, 62, 63-88-106-174]. Triploid plants are rare in nature because of their inviable seeds and resulting lack of progeny, so it is challenging to detect naturally occurring triploid plants [46, 48-53, 62, 63-88-106-157]. However, due to their faster growth and seedlessness, they will be useful for improving biomass, fruit and flower traits, and other qualities of economically important food, medicinal, bioenergy, and ornamental plants, reducing or eliminating the invasiveness of many crop and horticultural plants [46, 48-53, 62, 63-88-106-174]. So scientists have intentionally bred triploids through traditional and modern technologies[46, 48-53, 62, 63-88-106-157-174]. Natural selection, interploid sexual hybridization, endosperm culture, protoplast fusion were used for production of triploids [46, 48-55, 61, 62, 63-88-106-157]. A lot of plant species produced triploid plants and they have a popular application [46, 48-53, 62, 63-88-174]. There are more talks about interploid sexual hybridization and endosperm culture [46, 48-53, 62, 63-88-157]. As endosperm is a triploid tissue, it is thought that endosperm culture is the most direct and efficient method for production of triploid plants [46, 48-53, 62, 63-88-106]. Although endosperm culture is not yet entirely routine, many successful protocols have been developed over the last 15 years [46, 48-53, 62, 63-88-106-157]. In suitable media, 82% shoot and 80% root regeneration can be achieved from endosperm cultures of *Phlox drummondii* [46, 48-53, 62, 63-88-106]. Protoplast fusion technology has been utilized in many crops to generate allotetraploid somatic hybrids and sometimes triploids can be produced [46, 48-53, 62, 63-88-106-157]. It is important to combine the traditional methods with modern methods to promote development of breeding triploid[46, 48-53, 62, 63-88-106-174]. In the future, marker-assistant selection technique, which has already been used in grape breeding, together with embryo rescue technique will continuously play an important role in the breeding triploid plants[46, 48-53, 62, 63-88-106-174]. Other new strategies might be developed to induce triploid plants. With the rapid development of genomics research and advanced biology technologies, perhaps new methods to induce formation of triploids and new avenues of research into and using triploid plants will become possible [46, 48-53, 62, 63-88-106-157-161].

Triploid cannabis plants certainly boast some impressive benefits[46, 48-53, 62, 63-88-106-157]. They offer a seedless crop, bigger yields, large buds, and more phytocannabinoids [46, 48-53, 62, 63-88-106-161]. However, these cultivars largely remain confined to research laboratories for the time being[46, 48-53, 62, 63-88-106-157]. Plus, they also have some disadvantages. Their incredibly low fertility means that growers will have to indefinitely maintain a line of clones in order to maintain a progeny that they like[46, 48-53, 62, 63-88-106-157]. Inevitably, this demands resources such as space, lighting, and nutrients. On top of this, triploid varieties are hard to create [46, 48-53, 62, 63-88-106-157-161]. They require knowledge of plant genetics and the ability to create tetraploid strains as parent stock[46, 48-53, 62, 63-88-106-157-161]. Realistically, the vast majority of small-scale growers lack the resources and knowledge to make their own triploid genetics [46, 48-53, 62, 63-88-106-157].

6. Cannabis sativa: Tetraploid Plants

Tetraploid cannabis plants are similar to triploids in the sense that they are also polyploids [46, 48-53, 62, 63-88-106-174]. However, instead of three sets of chromosomes, each cell possesses four—two sets from each parent [46, 48-53, 62, 63-88-106-174]. These varieties are somewhat easier to create than triploid ones viewed them as a botanical precursor [46, 48-53, 62, 63-88-106-174]. Plant scientists have created tetraploid plants through chemical intervention [46, 48-53, 62, 63-88-106-174]. There are several molecules that, when applied to a weed plant, cause cells to multiply their chromosomes [46, 48-53, 62, 63-88-106-174]. These compounds include colchicine, a naturally occurring chemical found in autumn crocus that interferes with cell division [46, 48-53, 62, 63-88-106-174]. Researchers also used oryzalin, a less toxic alternative, to achieve the same result [46, 48-53, 62, 63-88-106-174]. Tetraploid cannabis varieties also possessed unique traits that make them attractive to breeders and growers [46, 48-53, 62, 63-88-106-

174]. These qualities include: **Unique leaf morphology:** Tetraploid varieties have different leaves compared to their diploid cousins. Their fan leaves are larger with longer and thicker leaflets. The guard cells that open and close the stomata are also thicker and longer[46, 48-53, 62, 63-88-106-174]. **Greater trichome density:** Trichomes are the small glands found on bud and leaf surfaces. These crystal-like structures are responsible for creating cannabinoids and terpenes, the chemicals responsible for the effects of each strain [46, 48-53, 62, 63-88-106-174]. Studies showed that tetraploid sugar leaves produce around 40% more trichomes than diploid ones. **Bigger yields:** Tetraploid plants produce bigger yields overall [46, 48-53, 62, 63-88-106-174]. This includes a slightly higher amount of dry bud and a significantly larger amount of leaf trim that can be used to make hash and other extracts[46, 48-53, 62, 63-88-106-174]. **More terpenes:** Aromatic terpenes are responsible for the unique scents and flavor of each strain. They also play an important role in the subjective effects of different cultivars[46, 48-53, 62, 63-88-106-174]. Early studies showed that tetraploid varieties produced far more terpenes in their leaves and flowers[46, 48-53, 62, 63-88-106-174]. Considering the impressive traits mentioned above, tetraploid cannabis clearly has a lot of potential[46, 48-53, 62, 63-88-106-174]. However, one could see them arrive on the market in the near future. Breeders are likely to use this technology to create strains that are more productive, resinous, flavorful, and potent[46, 48-53, 62, 63-88-106-174]. Chances are, one can see tetraploid versions of some of the most well-known cannabis strains emerge at some point[46, 48-53, 62, 63-88-106-174]. Crossing a diploid with a tetraploid results in a progeny with three sets of chromosomes[46, 48-53, 62, 63-88-106-174]. Although tetraploid plants exhibit decreased female fertility compared to diploids, they are still able to reproduce (they just create fewer seeds) [46, 48-53, 62, 63-88-106-174].

According to the study conduced by Parsons et al., (2019) [48], the effects of polyploidization on drug-type Medical *Cannabis sativa* strains is unknown [48]. Polyploidy can be induced through application of antimitotic agents to seeds, seedlings, in vivo shoot tips, or in vitro explants [48-53]. However, drug-type Medical *Cannabis sativa* strains are not genetically stable when propagated through seeds, and while there has been little success in regenerating cannabis shoots from callus [48]. The propagation of high THC drug-type Medical *Cannabis sativa* in tissue culture using nodal explants has been described by Parsons et al., (2019) [48, 54, 55]. These plants have been shown to be genetically and chemically stable through 30 rounds of tissue culture propagation [48, 54, 55]. A promising approach for cannabis breeding involves the process of polyploidization, in which the number of copies of each chromosome is increased.

Much like triploid genetics, tetraploid plants display some seriously impressive traits, and they are relatively easy to create using chemical manipulation[46, 48-53, 62, 63-88-106-174]. However, they also have a few disadvantages. First, their low fertility makes successful crosses hard to accomplish [46, 48-53, 62, 63-88-106-174]. They also appear to have a slower rate of growth compared to diploid varieties, making them less appealing to growers that value speed[46, 48-53, 62, 63-88-106-174]. Finally, for now, they remain confined to a niche area of breeding, and a lack of understanding surrounding tetraploids will make them tough to work with for the most amateurs[46, 48-53, 62, 63-88-106-174].

Although just a single species of plant, a gigantic industry revolves around cannabis. What started as a wild plant has, with the help of human innovation, become a diverse domesticated plant that takes on many different forms. Breeding breakthroughs have ushered in the likes of autoflowering and feminized strains, as well as the first true F1 hybrids[46, 48-53, 62, 63-88-106-174]. Now, it looks like polyploids are next in line to shake up the industry [46, 48-53, 62, 63-88-106-175]. The cannabis breeding has reached the point of multiplying the amount of chromosomes in plant cells [46, 48-53, 62, 63-88-106-175]. Both triploid and tetraploid cannabis present some promising advantages, including bigger buds, more THC, and more terpenes[46, 48-53, 62, 63-88-106-175]. Research remains in the early stages, but one can expect to hear much more about polyploid cannabis in the coming years[46, 48-53, 62, 63-88-106-175].

7. Conclusion

Cannabis sativa L. belongs to the family *Cannabaceae* is a dioecious plant, producing male and female flowers on separate unisexual individuals. The ploidy level of plants is associated with morphological and biochemical characteristics, and its modification has been used as a strategy to alter the quantitative and qualitative patterns of secondary metabolite production in different medicinal plants including cannabis. Different approaches either chemical (colchicine, oryzalin, caffeine, trifuralin, or phosphoric amides) or gaseous i.e. nitrous oxide have been deliberated as strong polyploidy causing agents in plants. Polyploidization can be induced by many anti-mitotic agents, among which colchicine, oryzalin, and trifluralin are the most common. Oryzalin is a potent herbicide that inhibits microtubule polymerization to promote polyploidization. The cannabis breeding has reached the point of multiplying the amount of chromosomes in plant cells. Both triploid and tetraploid cannabis present some promising advantages, including bigger buds, more THC, and more terpenes. Triploid cannabis plants certainly boast some impressive benefits. They offer a seedless crop, bigger yields, large buds, and more phytocannabinoids. Therefore, unintended cross-pollination of *Cannabis sativa* crops is one of the most important threats to cannabinoid production and has been shown to reduce

cannabinoid yield. Ploidy manipulation has been used in other crops to improve agronomic traits and reduce fertility. One potential solution good for both the cannabis industry and for the environment is to create seedless versions of plants that have been shown to be, or that have potential to be invasive. Thus, seedless triploid cannabis varieties can play an important role in neutralizing the invasiveness of introduced plants and blocking the cross pollination.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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