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Cannabis sativa: Monoecious species and Hermaphroditism: Feminized seed production- A breeding effort

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Abstract

Cannabis sativa L. is a dioecious wind-pollinated, although monoecious plants (male and female flowers on same plant) can occur in some population or developed by breeding efforts. Monoecious species have many advantages in the agricultural and industrial sectors due to the self-pollination and minimum variation in cannabinoid content from generation to generation. Monoecious species of cannabis also play an important role in the production of feminized seed production. Production of all female seed requires induction of female plants to develop male flowers that produce genetically female pollen. The aim of the cannabis commercial growers would like to access the feminized seed to produce all-female crops. Exogenous application of plant growth regulators can modify or reverse sex morphology in plants. There are a number of solutions that can be sprayed on female plants to create male pollen sacs: benzothiadiazole, gibberellic acid, silver thiosulphate, silver nitrate, and colloidal silver. Silver thiosulfate (STS) is the most effective compound for inducing sex reversal and feminizing seeds in high-THC cannabis cultivars. A hermaphrodite flower is one that has both staminate (male) and pistillate (female) floral structures on the same plant. A hermaphrodite flower is in the form of banana-shaped male anthers that emerge from the female flowers in small bunches, changing from green to a pale yellow as they develop. However, plants grown from feminized seeds are more likely to become hermaphrodites. The development of male anthers within the female inflorescence is known as hermaphrodite. Hermaphroditism is due to physical or chemical stress.

Keywords: Banana male anthers; *Cannabis sativa*; Dioecious; Feminized seeds; Hermaphrodite flower; monoecious; Sex determination; Silver Thiosulfate (STS)

1. Introduction

Cannabis sativa L. belongs to the family *Cannabaceae* is a dioecious plant, producing male and female flowers on separate unisexual individuals [6-8, 37-43, 86, 97, 98, 101, 125- 129]. *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 [1-43, 125-129]. Therefore, a breeder can produce monoecious cannabis plants [6-8, 37-43]. Production of all female seed requires induction of female plants to develop male flowers that produce genetically female pollen [44]. The aim of the cannabis growers would like access to feminized seed to produce all-female crops [44]. 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 [1-43]. Male plants die shortly after flowering [1-35-43]. The female plants live 3 to 5 weeks until seed is fully riped [1-35-43]. Therefore, the plants are obligatory out-crossers [1-45]. According

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to Timoteo Junior and Oswald (2024) [68], with the partial legalization of high-THC *Cannabis sativa* across 23 US States for recreational use and 38 US States for medical purposes in the United States, the cannabis industry is poised for significant growth [68]. Projected to reach a sales volume of $50.7 billion by 2028, this growth is driven by the trend of lifting cannabis prohibition [68]. High-THC *Cannabis sativa* cultivars, containing more than 0.3% Λ-9 tetrahydrocannabinol (D9-THC) as defined by the 2018 US Farm Bill, are used for both medicinal and recreational purposes [68].

Although both male and female plants are capable of producing cannabinoids in equal concentrations, female plants produce greater floral biomass than male plants and thus are exclusively used in commercial Medical *Cannabis sativa* (drug or marijuana) production facilities [1-43, 86, 97, 98, 101, 128]. One male cannabis plant can ruin the entire female plant crop due to uncontrolled pollination and crop is designated as contaminated [6-8, 39-40]. Moreover, after pollination, female plants alter their relative investment in phytochemicals by reducing the production of secondary metabolites like cannabinoids, flavonoids, and terpenoids [6-8, 39-40]. Fertilized female cannabis plants generate smaller flowers with significantly less terpene, flavonoid, and cannabinoid content than unfertilized females [1-44]. This is because when female cannabis plants undergo an extended period of virginity they will actively increase the number and size of their female sex organs, resulting in larger, more plentiful buds [45-46]. This increase in pollen-receptive tissue seems to be an evolutionary adaptation designed to make it more likely that the females will fertilize in scenarios when males are extremely uncommon [45-46]. Today, most cannabis growers take advantage of this adaptation and take steps to prevent fertilization and maximize yields [45-46]. They do this by isolating female cannabis plants from males or they buy feminized seeds, which are specially bred to only produce female plants [45-46]. Therefore, early diagnosis of sex is very important to both breeders and farmers for cannabis crop improvement or production purposes [6-8, 39-40, 84-120]. Cannabis sex determination could be modified by applying exogenous growth regulators or chemicals, which can influence the ratio of endogenous hormones and hence the incidence of sex organs [6-8, 39-45]. Silver compounds such as silver nitrate ($AgNO_3$) or silver thiosulfate ($Ag_2S_2O_3$; STS) have been found to have masculine effects in many plant species including cannabis [6, 8, 39-45].

Early diagnosis of sex is very important to both breeders and farmers for cannabis crop improvement or production purposes [6, 8, 39-40, 84-120]. Cannabis is anemophilous (wind-pollinated). For instance, in *Cannabis sativa*, if pollen is released near un-pollinated female plants, the crop of exclusively female plants will be pollinated and is considered by federal regulators in Canada as Contaminated [6, 8, 39-40]. Therefore, unfertilized female plants were only used for the commercial production of Medical *Cannabis sativa* (drug or marijuana) [6, 39-40]. In Canada, pollinated cannabis can only be used for oil extract, not raw floral material, limiting market capacity [39-40]. Moreover, pollinated cannabis has lower cannabinoid content, making it a less efficient way to produce oil extracts [39-40]. Moreover, after pollination, female plants alter their relative investment in phytochemicals by reducing the production of secondary metabolites like cannabinoids, flavonoids, and terpenoids [6, 8, 39-40]. In the absence of pollen, stigmas on female plants continue to grow and thus produce more surface area on which cannabinoids can be produced [6, 8, 39-40]. Because of this **negative impact of pollination** on cannabinoid yield, cannabis industrial growers rarely maintain male plants in production facilities, and instead propagate their stock of female plants by vegetative cloning [6, 39-40]. Since pollination has significant negative consequences for cannabinoid production, a lack of knowledge of sex can be costly for the farmer in this expanding agricultural business, providing practical reasons for exploring this information [6, 8, 39-40]. The study conducted by Campbell et al., 2021 [39] reported that males grew significantly longer hypocotyls than females by week 2, but this difference is depended on the cultivar investigated [39-40]. Preliminary evidence suggested that co-sexual plants may be distinguished from male and female plants using short hypocotyl length and seedling height, although this relationship required more study since sample sizes of co-sexual plants were small [39-40]. In one of the cultivars, two-week old male plants tend to produce longer hypocotyls than other plants, which may help to identify these plants prior to anthesis [39-40]. The preliminary data suggests that short hypocotyl length may be an indicator of co-sexuality [39-40].

Cannabis sativa has been used for thousands of years for recreational, medicinal, or religious purposes. *Cannabis sativa* and *Cannabis indica* are the native of Indian origin found as wild noxious weed in the foothills of Indian Himalayan Region and other parts of India, China, Nepal, Bhutan, Sri Lanka, Pakistan, Afghanistan, Persian, Iran, and Morocco and plains of Pamir (a high mountain range centered in eastern Tajikistan with extensions into Afghanistan, the Republic of China and Kyrgyzstan) [1-35, 123]. *Cannabis sativa* is cultivated as a crop in different regions of India but it is also found as a weed in different crops [125-127]. It is a common weed species of different kharif crops (Dhillon, 2024) [125-127]. It is also documented as a weed species in wheat crop (rabi crop) fields in the state of Punjab, India [125-127].

Now a days *Cannabis sativa* is a globally domesticated, cultivated and introduced species occurring in North and South America, Europe, Africa, Australia, Asia and other parts of world [1-35]. These cannabis species are hybrid varieties and known for very high levels of THC (0.3 to 38%) as compared to wild noxious weed found in all the parts of India [1-35].

On the other hand, *cannabis* interbreeding has contributed to the enormous phenotypic and chemical diversity of *Cannabis* cultivars that are in use today [1-35, 128]. There has been an increased interest in medical applications of cannabis over the last decades [1-40]. Cannabis can be classified based on genetics, phenotypic properties, and chemical composition [1-40]. All these types are rich in bioactive phytochemicals [1-40]. However, the phytochemical composition varies in different types [1-40].

The important characteristic of *Cannabis sativa* L. is the production of phytocannabinoids in abundance which are present in their acidic form in plant tissue [1-46, 101, 125]. Phytocannabinoids are produced as a part of defensive mechanism in the trichomes on flower bracts of female inflorescences [1-40]. Cannabis contains a diverse array of chemical compounds, including cannabinoids, which are responsible for its therapeutic effects [1-35, 124]. The concentration and composition of cannabinoids varies between cannabis cultivars, leading to distinct chemical profiles and differential biological effects [1-35, 124]. In addition to cannabinoid content, morphological traits play a crucial roles in the overall physiology, yield potential, and quality of cannabis plants [1-35, 124, 125]. For regulatory and agronomic purposes, *C. sativa* plants are classified based on the level of the phytocannabinoid intoxicant, Λ9-tetrahydrocannabinol (Λ9-THC) [18].

In recent years, the medicinal applications of *Cannabis sativa* L. have gained wider attention worldwide [1-46, 124, 125-129]. The female *Cannabis sativa* flowers have densely packed glandular structures called trichomes that store the phytocannabinoids, tetrahydrocannabinolic acid (THCA) and Cannabidiolic acid (CBDA) which must be decarboxylated by heat to produce Δ9-tetrahydrocannabinol (THC: intoxicating) and Cannabidiol (CBD: non-intoxicating) [1-35]. The two cannabinoids the most well known for their therapeutic properties are, Δ9-tetrahydrocannabinol (THC) and Cannabidiol (CBD) [1-35-45]. THC and CBD are the neutral homologs of tetrahydrocannabinolic acid (THCA) and Cannabidiol acid (CBDA) respectively [1-35-45]. A conventional classification model of cannabinoids is due to their chemical contents dividing them to eleven subclasses including Cannabigerol (CBG), Δ9-tetrahydrocannabinol (Δ9-THC), Cannabidiol (CBD), Cannabichromene (CBC), **Cannabinol** (CBN), (−)-Δ8-transtetrahydrocannabinol (Δ8-THC), Cannabicyclol (CBL), Cannabinodiol (CBND), Cannabielsoin (CBE), Cannabitriol (CBT) and miscellaneous [1-35-45-129].

2. *Cannabis sativa*: Monoecious species

The use of the cannabis plant as a source of therapeutic compounds is gaining great importance since restrictions on its growth and use are gradually reduced throughout the world[1-46-80]. Intensification of medical (drug type) cannabis production stimulated breeding activities aimed at developing new, improved cultivars with precisely defined, and stable cannabinoid profiles [1-46-80]. *Cannabis sativa* L. is an annual, predominantly dioecious (male and female flowers occur on separate plants), rarely monoecious (male and female flowers occur on the same plant) plant [1-46-80]. It is wind pollinated and flowers under short day conditions [1-46-80, 101]. *C. sativa* research mainly focuses on dioecious plants compared to monoecious plants [1-46-80]. The monoecious plants evolved due to natural mutation in dioecious *C. sativa* [1-46-80]. The dioecious plant undergoes cross-pollination, which leads to the variation in cannabinoid content from generation to generation [1-46-80, 101]. Monoecious species have many advantages in the agricultural and industrial sectors due to the self-pollination and minimum variation in cannabinoid content from generation to generation [1-46]. Cannnabis monoecious species also play an important role in the production of feminized seed production [41, 44-80]. The solution of silver thiosulfate at 3 mM is applied to selected branches of the cannabis female plant, causing male flowers to develop within 1-2 weeks [44]. These male flowers are then used to pollinate other female plants, resulting in feminized seeds [37, 38, 41, 44-46]. The cannabis grower in the commercial production uses only feminized seeds for the growth of female plants [44]. Monoecious individuals are found with hermaphrodite flower or bisexual inflorescence [38, 47, 48-80]. The hermaphrodite inflorescences consist of few to many male flowers and female flowers within the leaf axils [38, 49-50]. The previous studies of Punja and Holmes (2020) [37] demonstrated that the female flowers develop early hermaphrodite inflorescence, following the development of the male flowers after four weeks of the flowering stage [37]. The induction of hermaphroditism, sexual expression of progenies and the phytocannabinoid content were affected by several abiotic factors like low temperature, altered photoperiods, hormonal or chemical treatments and soil nitrogen status [37, 38, 45, 46]. *C. sativa* is a dioecious plant with highly cross-pollinated behavior which may lead to variation in cannabinoid content and an increase in Λ9-THC in seed progenies [1-38-46]. Such a variation in cannabinoid content can be minimized in monoecious *C. sativa* due to its self-pollinating nature [37, 38]. Such monoecious plants can maintain cannabinoid content in subsequent generations [37, 38]. Earlier studies demonstrated that the morphological characteristics like plant height, stem diameter, biomass and fiber yield showed extensive variation among the accessions of both monoecious and dioecious hemp [38]. Ghosh et al., (2023) [38] also observed significant morphological variation in leaf and female floral characters in the Indian monoecious population ($P < 0.05$) [38]. Further the study by Ghosh et al., (2023) also confirmed that heatmap cluster analysis also demonstrated the distinctive characteristics in monoecious accessions, particularly the highest stem diameter, leaf length, leaf width, internodal length, and canopy diameter in CH-1 accession [38]. In *C. sativa*, the phytocannabinoids are synthesized and accumulated in the glandular trichomes distributed on the aerial parts, i.e., leaves, stem and inflorescence [1-38-45]. The earlier detailed studies on dioecious *C. sativa* reported three types of glandular trichomes, i.e., capitate sessile, capitate stalk and bulbous [1-38-50]. The study by Ghosh et al., (2023) [38] revealed that the organ-specific different glandular trichomes size in monoecious plants [38]. Furthermore, the study by Ghosh et al., (2023) [38] reported that the capitate-sessile and capitate-stalked glandular trichomes are PCS glands in monoecious plants [38]. The study by Ghosh et al., (2023) [38] confirmed that monoecious plants contained a maximum quantity of phytocannabinoids with the highest antioxidant activity [38]. Likewise, phytocannabinoids synthesizing glands are more in bract of female flowers of monoecious accessions [38]. The study by Ghosh et al., (2023) [38], also observed that monoecious accessions of Indian origin can be used in the medical cannabis industry with high THC content [38]. Moreover, it could be easily maintained as a uniform chemotype from generation to generation with the help of self-pollination [38]. The levels of phytocannabinoids in different organs can be exploited in pharmaceutical-nutraceutical-based products that could be useful for industries [38]. All the obtained results of the study conducted by **Ingallina et al., (2020)** [121] indicated that each monoecious cultivar has a characteristic chemical profile that changes during the season[121]. Indications of the levels of specific compounds responsible for sensorial and/or pharmaceutical-nutraceutical properties could be useful for the industries which use *Cannabis sativa* L. based products [121]. The study of **Baldini et al., (2018)** [122] confirmed that the monoecious hemp cultivars available today allowed a multipurpose exploitation of the crop[122]. In fact, the same crop could supply inflorescences (essential oil, phytocannabinoids, etc.), seeds (vegetable oil and meal), and stems (industrial application), improving crop sustainability [122]. This could encourage the completion of the supply chain at a local level, including investments in first-processing technologies [122].

Cannabis sativa is a diploid species with **2n = 20** and genome size estimated at 818 Mb and 843 Mb for female and male plants, respectively [51, 38, 89, 90,129]. According to the karyotype of the species, female plants are homogametic (XX), and males are heterogametic (XY) [52, 53, 38]. 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) [54]. In this species, the karyotype consists of nine autosomes and a pair of sex chromosomes (X and Y) [51]. Female plants are homogametic (XX) and males are heterogametic (XY), with sex determination controlled by an X-to-autosome balance system [53]. The estimated size of the haploid genome of C. sativa is 818 Mb for female plants and 843 Mb for male plants, owing to the larger size of the Y chromosome [51, 89, 90]. Monoecious plants have several agricultural advantages over dioecious ones, such as seed yield, higher crop homogeneity and synchronized maturity [38, 55]. Monoecious plants can undergo self-pollination and minimize genetic variation [38, 55]. It has been observed that the sexual phenotype of monoecious plants is unstable [38, 55]. The multiplication of monoecious seeds in the next generation produces dioecious male and female progenies [38, 55].

Plant growth hormones such as ethylene, auxins, and cytokinin promote the development of female flowers in cannabis, while gibberellins stimulate the formation of male flowers; controlling these phytohormones can partially reverse plant sex [37, 38, 39, 41, 44, 56-64, 68-72-80, 99, 108]. Exogenous application of plant growth regulators can modify or reverse sex morphology in plants [37, 38, 39, 41, 56-80]. In hemp, auxins, ethylene, and cytokinins promote the formation of female flowers on male plants; gibberellins promote the formation of male flowers on female plants [37, 38, 39, 56,58, 68-72-80]. Mohanram and Sett (1982) [64] induced the formation of male flowers on female hemp plants using silver nitrate and STS, which inhibit the action of ethylene [37, 38, 64]. Monoecious hemp cultivars demonstrated greater seed yields and greater crop homogeneity, and are easier to harvest mechanically than dioecious plants [37, 38]. Sex expression in monoecious hemp is not as well understood as for dioecious plants, but it is believed to be a heritable trait present on the X chromosome or autosomes [65, 66]. It has also been suggested that the monoecious state originated from a small translocation from the Y chromosome, or by one or several mutated genes [65, 66].

According to the study reported by **Lubell and Brand** (2018) [44], the male flowers can be produced easily and consistently on female plants through application of foliar sprays of STS under short-day conditions [44]. The study reported by Lubell and Brand (2018) [44] confirmed that Silver thiosulfate (STS) at 3 mM was the most efficacious treatment for all strains [41, 44]. The majority of inflorescences had 100% male flowers at 3 mM STS, and terminal inflorescences had 95% conversion to male flowers [44]. Silver thiosulfate at 0.3 mM produced partial conversion to male flowers, whereas most inflorescences had around 50% male flowers, except for CBD hemp A, which demonstrated greater levels of masculinization [44]. At 0.3 mM STS, terminal inflorescences of CBD hemp A had 91% conversion to male flowers [44]. Therefore, producers and breeders should be able to masculinize female hemp plants routinely by using short-day conditions of 8 h and three foliar sprays of 3 mM STS at weekly intervals [44]. Further, this method will be applicable for a broad range of genetically diverse hemp genotypes [44]. Pollen produced by male flowers on genetically female plants can be used to produce all-female seed, but growers and breeders should be aware that pollen output may be reduced compared with pollen output from genetically male plants [44].

The general practice behind feminization is that *Cannabis sativa* female plants are forced to produce pollen, which is in turn used to pollinate other female plants [41, 44, 67, 71]. Resulting seeds will be feminized, with no risk of further pollination [44,45, 46 67]. Applications that reduce the ethylene level in tissues or antagonise the action of ethylene causes the formation of male flowers instead of female ones [44, 67, 71]. There are a number of solutions that can be sprayed on female plants to create male pollen sacs: benzothiadiazole, gibberellic acid, silver thiosulphate, silver nitrate, and colloidal silver [37-67].

Colloidal silver is the easiest to source or make. It is non-toxic, non-caustic, and can be bought from a pharmacy or easily online [67, 71]. The other solutions can be dangerous, difficult to get a hold of, and expensive—except gibberellic acid, which can be found in nurseries, but is not as effective as colloidal silver [67, 71]. Colloidal silver is a distilled water-based solution in which microscopic particles of silver are suspended [67, 71]. The nature of colloids means the particles will never settle out and can not be removed by normal filtering [67, 71]. Colloidal silver is available commercially, or one can make own [67, 71]. It has numerous uses as an alternative medicine [67]. For example, it is used to soothe burns, as an antiseptic and digestion stimulant in people, and as a fungal control in horticulture [67, 71]. Feminizing clones is the usual practice as the growth, flowering, and resin characteristics from the mother are already known [67, 71]. Plants can be induced to grow male sex organs as late as four weeks into flowering [67, 71]. Though spraying one week prior to the light changeover is recommended for clones [67, 71]. If a plant grown from seed is being used, wait until the plant has sexed before spraying so you can be sure it is female [67, 71].

Spray the plants to be feminized with colloidal silver every day, and three times a day if grower can manage [67] Soak them well [67] Do this for two weeks, then leave the plants to grow as normal [67] Some growers reported getting results after spraying for only 5–10 days [67], When sexing begins, male pollen sacs will develop instead of female calyxes and pistils [67]. Male plants mature much faster than females, and viable pollen can be expected within 3–4 weeks once the plant has been sexed [67]. Some growers will spray until the plant shows sexual growth, just to be sure the method has worked [67]. Make sure these plants are well-isolated from any flowering females [67] A burst pod can release millions of pollen spores, and it only takes one spore per hair to create a seed [67]

Sinsemilla is an unnatural state for cannabis [67]. Without human intervention, it would be rare to find an unpollinated female in the wild—unless it was sterile [67]. When sinsemilla plants are left to go beyond their desirable maturation stage by a number of weeks, the plant, through whatever amazing processes evolution has bestowed, knows it has not been pollinated [67]. As a last ditch effort at propagation, cannabis plant will produce male pollen sacs in an effort to self-pollinate. This is not the result of genetic or stress-induced hermaphroditism [67]. They are genuine XX chromosome female bananas[67]. With all the genetic information from the female and no Y chromosome, using rodelized pollen creates female-only seeds, although as with colloidal silver, an occasional male may appear [67].

Several studies with hormonal manipulation confirmed gender reversal in *Cannabis. sativa* L. and proved bi-potency of sexually predetermined dioecious cannabis plants [37, 38- 45, 46-74, 129]. It has been shown that **gibberellins** induce **maleness** in plants, while ethylene, cytokinins, and auxins stimulated the formation of female flowers on genetically male plants [37, 38- 45, 46-74]. The effects of several exogenous substances, known to be involved in sex expressions, such as silver thiosulfate (STS), gibberellic acid (GA_3), and colloidal silver, were analyzed [37, 38- 45, 46-74]. In one of the experimental study reported by Flajšman et al. (2021) [41] various concentrations of silver thiosulfate (STS), gibberellic acid (GA_3), and colloidal silver were tested within 23 different treatments on two high cannabidiol (CBD) breeding populations (1-17, 18-52). Experimental results of Flajšman et al. (2021) [41] showed that spraying whole plants with silver thiosulfate (STS), once is more efficient than the application of silver thiosulfate (STS) on shoot tips while spraying plants with 0.01% GA_3 and intensive cutting is ineffective in stimulating the production of male flowers [37, 41]. Additionally, spraying whole plants with colloidal silver was also shown to be effective in the induction of male flowers on female plants, since it produced up to 379 male flowers per plant [37-41-46]. Cannabis sex determination could be modified by applying exogenous growth regulators or chemicals, which can influence the ratio of endogenous hormones and hence the incidence of sex organs (15-51). Silver compounds such as silver nitrate ($AgNO_3$) or silver thiosulfate ($Ag_2S_2O_3$; STS) have been found to have masculine effects in many plant species, e.g., in *Coccinia grandis*, *Cucumis sativus*, *Silene latifolia* , *Cucumis melo*, and also *Cannabis sativa* [37-40, 99, 108].

In one of the experiment reported by Mohanam and Sett (1982) (35) applied 50, 100, and 150 mg of silver nitrate and 25, 50, and 100 mg of silver thiosulfate (STS) to shoot tips of female cannabis plants [37, 56-64]. Both silver compounds successfully evoked the formation of male flowers, but STS was more effective than $AgNO_3$ [37, 56-64]. 100 mg of STS caused the highest number of fully altered male flowers, which was significantly higher than the number of reduced male, intersexual, and female flowers [37-56-64]. On the other hand, the treatment of shoot tip with 100 mg of $AgNO_3$ resulted in more than half the lower number of male flowers, with the highest amount of $AgNO_3$ (150 mg) being ineffective in altering sex expression [37-56-64]. Furthermore, pollen from all induced male flowers was viable in vitro and also successfully induced seed set [37, 56-64]. More recently, two other studies have also successfully used silver thiosulfate (STS) to induce male flowers [37, 56-64]. Di-Matteo et al. (2020) [71] sprayed 3 mM of silver thiosulfate (STS) until runoff three times at 7- day intervals after exposing the plants to short-day conditions for 12 h (1-51). Adal et al. (2021) [75] applied 20 ml of silver thiosulfate (STS) (2.5 mg/ml) to whole plants on the first and third day after the start of 12-h lighting and fertilization on a foliar basis [75]. Foliar spraying of male cannabis plants with 960 ppm 2-chloroethane-phosphonic acid caused the highest formation of the fertile female flower [56-64]. A total of 100 mg/plant of cobalt chloride applied to the shoot tip triggers male sex expression in the female plants of Cannabis [37-56-64]. The mode of action of these chemicals in plants is not yet entirely deciphered [37-75].

3. *Cannabis sativa*: Feminized Seed Production

Cannabis sativa is a short day, dioecious, annual plant, where female plants are favored for THC production, which requires seed feminization techniques to ensure an accurate female plant population [37-44, 56-64, 68-71]. This involves using an ethylene inhibitor to induce sex reversal, leading to male flower development on female plants, allowing for self-pollination and the production of feminized seeds [37-44, 56-64, 68-71]. However, challenges such as seed viability and the occurrence of male flowers in progeny have been noted [68]. Literature findings indicated that silver thiosulfate (STS) is the most effective ethylene inhibitor for sex reversal and seed feminization in high-THC cannabis cultivars [44, 68]. Specifically, a single dose of 3 mM STS should be applied during the vegetative stage via foliar spraying until runoff, followed by exposure to a short photoperiod of up to 12 hours to induce flowering and seed production [41, 44, 68]. Timoteo Junior and Oswald (2024) [68] are of the opinion that progeny plants should be assessed for seed germination rate and compared for growth performance with the original parent plant to assess the declining effects of inbreeding [68]. Adhering to these guidelines can improve the quality and viability of feminized seeds, meeting commercial market standards and industry demands for high-THC Cannabis cultivars [68].

Manipulation of sex expression is of paramount importance in breeding medical cannabis, since only genetically and phenotypically female plants are used in commercial cultivation [37-44, 56-64, 68-71]. It enables self-pollination and crossing of female plants for obtaining pure lines and feminized seeds, respectively [37-44, 56-64, 68-71]. Upon germination, the latter produce entirely female progeny that is used for the production of female flowers [37-44, 56-64, 68-71]. Most cannabis sex manipulation studies are performed on fiber-type hemp, and knowledge about the efficiency of various exogenous factors and application methods for inducing sex conversion in medical cannabis is needed [37-44, 56-64, 68-71].

Feminized seeds produce exclusively female plants with all female flowers, achieved by applying an ethylene inhibitor to female plants [37-44, 56-64, 68-71]. This induces sex reversal, prompting the development of male flowers on the female plants [41, 68, 69, 70]. Ethylene, a naturally occurring ripening phytohormone, typically promotes the formation of female flowers [37-44, 68-72]. However, when female cannabis plants are treated with ethylene inhibitors such as amino-ethoxy-vinyl-glycine (AVG), cobalt nitrate (CBN), colloidal silver, silver thiosulfate, 1-Methylcyclopropene (1-MCP), they undergo sex reversal, becoming monoecious or bisexual [37-41, 44, 56-64, 68, 69, 70-72]. Therefore, they bear both male and female flowers on the same plant, facilitating self-fertilization and seed production [37-41, 44, 56-64, 69, 70, 71, 72]. The resulting seeds exclusively carry the XX female chromosome inherited from the original self-crossed female parent, ensuring that all plants derived from these seeds are exclusively female, capable of producing all-female flowers plant [37-41, 44, 68, 69, 70, 71, 72]. For instance, Flajšman et al. (2021) [41], investigated the impact of foliar application of silver thiosulfate and colloidal silver on sex reversal and masculinization of female cannabis plants, resulting in the induction of male flowers, self-pollination of the remaining female flowers, and production of feminized seeds [37-41, 44, 56-64, 69, 70, 71, 72]. Additionally, apical application of amino-ethoxy-viny-lglycine (AVG) promoted the development of male flowers in lateral branches of dioecious female plants [63]. Sex reversal in cannabis plants is not exclusive to female plants; male plants can also undergo sex reversal by inducing female flowers for seed production (Moon et al., 2020) [73]. However, the offspring will exhibit male plants due to the inheritance of the parental heterogametic Y chromosome (Moon et al., 2020) [73]. For instance, male plants treated with ethephon foliar spray developed female flowers [73].

Literature research indicated that silver thiosulfate (STS) is the most effective compound for inducing sex reversal and feminizing seeds in high-THC Cannabis cultivars [37-41, 44, 56-64, 69, 70, 71, 72]. Specifically, applying a single dose of 3 mM silver thiosulfate (STS) during the vegetative stage by foliar spraying until runoff, followed by exposure to a short photoperiod of up to 12 hours, effectively induces flowering and seed production [37-41, 44, 56-64, 69, 70, 71, 72]. Progeny plants should be evaluated for germination rates and compared to the original parent plant for growth traits, such as plant height, biomass, and Λ-9-tetrahydrocannabinol (THC) yield, to assess the negative effects of inbreeding [37-41, 44, 56-64, 69, 70, 71, 72]. Following these guidelines will help growers and breeders enhance the quality and viability of feminized seeds, thereby meeting the high standards and demands of the commercial market for high-THC Cannabis cultivars [37-41, 44, 56-64, 69, 70, 71, 72].

4. *Cannabis sativa*: Hermaphroditic inflorescences

A hermaphrodite flower is one that has both staminate (male) and pistillate (female) floral structures on the same plant [37, 38, 45, 46, 74, 94]. However, despite growers' best efforts, there are rare cases where female cannabis plants develop male sex organs, which can produce pollen [37, 38, 45, 46, 74]. These hermaphrodite plants, also called "herms" or "hermies", will produce predominantly female flowers [37, 38, 45, 46, 74, 94]. However, within the buds emerge **banana-shaped anthers** which can produce and release pollen [37, 38, 45, 46-82, 94]. Cannabis plants herm in response to stress [37, 38, 45, 46, 74]. It is an evolutionary adaptation designed to be a last-ditch effort for reproduction in situations where the plant is in danger [37, 38, 45, 46]. Medical *Cannabis sativa* (drug or Marijuana type) plants are grown commercially for their psychoactive compounds, which are produced in the trichomes that develop on flower bracts in female inflorescences [1-60]. On occasion, it has been observed that hermaphroditic inflorescences can develop spontaneously [37, 38, 45, 46, 74]. The hermaphrodite inflorescences consist of few to many male flowers and female flowers within the leaf axils [37, 38, 45, 46, 74]. The previous studies demonstrated that the female flowers develop early in the hermaphrodite inflorescence, following the development of the male flowers after four weeks of the flowering stage [37, 38, 45, 46, 74]. The induction of hermaphroditism, sexual expression of progenies and the phytocannabinoid content were affected by several abiotic factors like low temperature, altered photoperiods, hormonal or chemical treatments and soil nitrogen status [37, 38, 45, 46, 74]. The development of male sex organs within the buds themselves is the primary indicator of hermaphroditism in cannabis plants [37, 38, 45, 46, 74]. These come in the form of **banana-shaped male anthers** that emerge from the female flowers in small bunches, changing from green to a pale yellow as they develop [37]. Cannabis growers can reduce the likelihood of a hermie plant if they can prevent the stresses [45, 46]. 1) Too-hot or too-cold growing environments. 2) Inconsistent start and stop timings for light cycles, as well as light leaks or other disruptions during the night. 3) Using lights that are too weak, too strong, or have a spectrum unsuitable for cannabis cultivation [45, 46]. 4) Placing lights too near or too far from plants. 5) Under watering, overwatering, overfeeding, or underfeeding. 6) Using subpar nutrients or applying them at the wrong time. 7) Root-bound plants, plants with rotted roots, or inadequately aerated growth media. 8) Defoliating too much, too frequently, or at the incorrect time from plants. 9) Diseases and pests [45, 46].

Certain cannabis varieties appear to be more sensitive to stress than others, which ay be due to an underlying genetic mutation [37, 38, 45, 46, 74]. However, that mutation has not yet been identified [37, 38, 45, 46, 74]. Some cannabis growers believed that plants grown from feminized seeds are more likely to become hermaphrodites [45, 46]. In fact, feminized seeds are created by treating female plants with a chemical, typically silver nitrate, that causes them to herm [45, 46]. Breeders then collect the pollen and use it to fertilize other female plants [45, 46]. The resulting seeds are all female because neither parent can pass along a Y-chromosome [37, 38, 45, 46, 74]. The belief is that the hermaphrodite parent that contributed the pollen passed along epigenetic traits to the offspring that make it more prone to herming [45, 46]. However, this theory is anecdotal, and it has not been verified in any peer-reviewed studies [45, 46]. Lastly, cannabis growers who let their plants flower too long also run the risk of having plants herm [45, 46]. Again, this is a last-ditch effort of the plants to reproduce [45, 46]. They have grown large buds in hopes of attracting pollen and now, sensing the end of their life, they are attempting to self pollinate [45, 46]. Hermaphroditism occurs widely in plants [37, 38, 39, 45, 46, 74]. Hermaphrodites occur abundantly throughout the plant kingdom with both stamens and carpels within the same flower [37, 38, 45, 46, 74]. Nevertheless, 10% of flowering plants have separate unisexual flowers, either in different locations of the same individual (Monoecy) or on different individuals (Dioecy) [37, 38].

These plants produce predominantly female inflorescences, but anthers (ranging from a few to many) may develop within the leaf axils or in pistillate flower buds [37, 38]. These hermaphroditic inflorescences can be induced by exogenous applications of different chemicals [37, 38-64], and by environmental stresses [37, 38, 45, 46], suggesting that external triggers and epigenetic factors may play a role [37, 38-46]. The hermaphrodite plants are functionally monoecious due to their ability to undergo self-pollination [37, 38]. Spontaneously occurring hermaphroditic inflorescences, in which pistillate flowers are accompanied by formation of anthers, leads to undesired seed formation; the mechanism for this is poorly understood [37, 38]. **The development of male anthers within the female inflorescence is known as hermaphrodite** [37, 38]. According to the study conducted by Punja and Holmes (2020) [37] , individual clusters of anthers appeared bright yellow and measured 2– 3 mm in length and were formed within the bract tissues and surrounded by stigmas [37]. In some cases, the entire female inflorescence was converted to a mass of anthers which emerged through the bracts [37, 38]. Formation of anthers within calyx tissues of female inflorescences is the characteristic of hermaphrodite [37, 38]. The study conducted by Punja and Holmes (2020) [37], beginning around week 4 of the flowering period, the appearance of individual anthers or clusters of anthers within the bract tissues adjacent to the stigmas was observed in hermaphroditic flowers at a frequency of 5–10% of the plants examined [37]. The anthers were visible in weeks 4–7 of the flowering period and were present until harvest [37]. In rare instances, the entire female inflorescence was converted to large numbers of clusters of anthers [37]. According to the study conducted by Punja and Holmes (2020) [37] , the spontaneous development of hermaphroditic inflorescences (pistillate flowers containing anthers) on female plants during commercial Medical *Cannabis sativa* (drug or Marijuana type) cultivation creates a problem for growers, since the resulting seed formation reduces the quality of the harvested flower [37]. The allocation of resources by the female plant to pollen production, followed by seed production, can result in disproportionately lower levels of terpenes and essential oils (by up to 56%) in the pollinated flowers compared to unfertilized female flowers [37]. Therefore, inflorescences containing seeds are of lower quality and frequently not suited for sale [37]. Un-pollinated female flowers, on the other hand, continue to expand growth of the style-stigma tissues, potentially to increase opportunities for attracting pollen, and consequently are more desirable commercially [37]. The study conducted by Punja and Holmes (2020) [37], also observed spontaneous formation of hermaphroditic flowers on 5–10% of plants of three different strains of Medical *Cannabis sativa* (drug or Marijuana type) grown indoors under commercial conditions [37]. In most cases, small clusters of anthers developed within certain female flowers, replacing the pistil [37]. In rare cases (two out of 1,000 plants), the entire female inflorescence was displaced by large numbers of clusters of anthers instead of pistils [37]. The factors which trigger this change in phenotype have not been extensively researched [37]. This is due, in part, to the restrictions placed by government regulatory agencies on conducting research experiments on flowering cannabis plants (including in Canada), which reduces the opportunity to conduct the types of controlled experiments that are needed to elucidate the basis for hermaphroditism [37]. In earlier research, induction of hermaphroditism in marijuana plants was achieved experimentally by applications of gibberellic acid [37-74]. Other studies showed that male and female flower ratios in marijuana plants could be altered by applications of chemicals such as 2-chloroethanephosphonic acid, amino-ethoxy-vinylglycine, silver nitrate, silver thiosulfate, or cobalt chloride [37-75]. Silver nitrate inhibits ethylene action in plants and was reported to increase male sex expression in marijuana, cucumber and gourd plants [37-75]. In a recent study, applications of silver thiosulfate induced male flower formation on genetically female hemp plants [37, 44]. These findings demonstrated the growth regulator levels in treated plants can impact hermaphroditic flower formation [37-74].

Physical or chemical stresses can also have a role in inducing staminate flower development on female plants of marijuana [37]. For example, external environmental stresses, e.g., low photoperiods and reduced temperatures in outdoor production, were reported to increase staminate flower formation [37]. Some plants formed hermaphroditic flowers when female plants were exposed to extended periods of darkness early during growth or during altered photoperiods during the flowering stage, although the exact conditions were not described [37, 76, 77]. Such stress factors could affect internal phytohormone levels, such as auxin:gibberellin ratios [78], which could in turn trigger hermaphroditic flower formation in marijuana plants [37-78]. In *Arabidopsis* plants, auxin, gibberellin and ethylene interact with jasmonic acid (JA) to alter stamen production [37, 79, 80]. Jsmonic-acid (A) production could potentially promote hermaphroditic flower formation but this requires further study [37, 79, 80]. Lability of sex expression may offer advantages in promoting seed formation in hermaphroditic plants subject to environmentally stressful conditions [37, 81]. The study conducted by Punja and Holmes (2020) [37], also observed a row of bulbous trichomes forming along the stomium on the anthers in staminate flowers and in hermaphroditic flowers, confirming earlier descriptions by Potter (2009) [82] and Small (2017) [74] for staminate flowers [74, 82]. The function of these trichomes is unknown [37, 74, 82]. The study conducted by Punja and Holmes (2020) [37] confirmed the visual observations of male flowers of marijuana indicated significantly more pollen was produced and released compared to hermaphroditic flowers [37].

The study conducted by Punja and Holmes (2020) [37], also reported that male plants released pollen over a period of 2–4 weeks; estimates are that each male flower can produce as many as 350,000 pollen grains [37]. While the proportion of hermaphrodites in populations of Medical *Cannabis sativa* (drug or Marijuana type) is unknown, the frequency of seed formation within the hermaphroditic flower during indoor production is likely greater, despite the lower amounts of pollen produced, compared to a female flower dependent on wind-dispersed pollen from a male plant (indoors or outdoors) [37]. The distance over which pollen is dispersed from individual anthers in hermaphroditic flowers is probably limited to a few meters in indoor or outdoor growing facilities, compared to up to 3–5 km from male plants grown under outdoor field conditions, depending on wind speed and direction [37]. The study conducted by **Punja and Holmes** (2020) [37] reported that male plants grow faster and are taller than female plants grown over the same time period, ensuring more rapid development of flowers and pollen dehiscence [37]. However, the complete exclusion of male plants in indoor Medical *Cannabis sativa* (drug or Marijuana type) production suggests that the majority of seed formed would be the result of selfing [37]. In outdoor cultivation of marijuana, where there could be several pollen sources, there is a greater likelihood of obtaining seeds that are the consequence of both self-fertilization and cross-fertilization [37]. When hermaphrodites were used as pollen donors, the sex ratio of offspring they produced through crosses was biased toward females [37].

The study conducted by Punja and Holmes (2020) [37] also observed that seeds collected from hermaphroditic flowers in indoor production gave rise to seedlings which expressed the female genotype in a PCR-based test, compared to an approximately 1:1 ratio of male: female plants from cross-fertilized seeds [37]. The study conducted by Punja and Holmes (2020) [37] reported male anthers and pollen production in hermaphroditic inflorescences of *Cannabis sativa* [37]. Formation of anthers within calyx tissues of female inflorescences of strain “Moby Dyck” grown under commercial conditions was also reported by Punja and Holmes (2020) [37]. This study also reported the clusters of anthers formed within the calyx tissues adjacent to the brown stigmas [37]. Pollen production and release from anthers along the line of dehiscence that appears as a longitudinal groove (stomium) has been noted by Punja and Holmes (2020) [37]. The spontaneous conversion of a female inflorescence to produce anthers and initial clusters of anthers forming within the calyx that normally surrounds the ovary [37]. Advanced stage of development of anthers in large clusters on the same plant was observed by Punja and Holmes (2020) [37]. Close-up of masses of anthers replacing the female inflorescence has been reported in hermaphroditic plants [37]. In one of the study reported by Punja and Holmes (2020) [37], the mature anthers that have become dried, and showed the prominent “banana-shaped” morphology in the female inflorescence is the characteristic of hermaphroditic cannabis plants [37]. The development of male sex organs within the buds themselves is the primary indicator of hermaphroditism in cannabis plants [37]. These come in the form of banana-shaped male anthers that emerge from the female flowers in small bunches, changing from green to a pale yellow as they develop [37].

5. Conclusion

C. sativa is a naturally dioecious plant with distinct male and female individuals and is rarely monoecious along with sexual dimorphism. It is an evolutionary adaptation designed to be a last-ditch effort for reproduction in situations where the plant is in danger. Monoecious species have many advantages in the agricultural and cannabis industrial sectors due to the self-pollination and minimum variation in cannabinoid content from generation to generation. The monoecious plants evolved due to natural mutation in dioecious *C. sativa.* Cannnabis monoecious species also play an important role in the production of feminized seed production. Feminized seeds produce exclusively from female plants with all female flowers, achieved by applying an ethylene inhibitor to female plants. This induces sex reversal, prompting the development of male flowers on the female plants. Ethylene, a naturally occurring ripening phytohormone, typically promotes the formation of female flowers. It has been shown that **gibberellins** induce **maleness** in plants, while ethylene, cytokinins, and auxins stimulated the formation of female flowers on genetically male plants. Most of the studies showed that male and female flower ratios in marijuana plants could be altered by applications of chemicals such as 2-chloroethane-phosphonic acid, amino-ethoxy-vinyl-glycine, silver nitrate, silver thiosulfate, or cobalt chloride. Silver nitrate inhibits ethylene action in plants and was reported to increase male sex expression in marijuana, cucumber and gourd plants. In a recent study, applications of silver thiosulfate induced male flower formation on genetically female hemp plants. These findings demonstrated the growth regulator levels in treated plants can impact hermaphroditic flower formation. Hermaphrodites occur abundantly throughout the plant kingdom with both stamens and carpels within the same flower. The development of male sex organs within the buds themselves is the primary indicator of hermaphroditism in cannabis plants. These come in the form of **banana-shaped male anthers** that emerge from the female flowers in small bunches, changing from green to a pale yellow as they develop.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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